OUTCOMES AND OPTIMAL TREATMENT OF PATIENTS WITH ACROMEGALY

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Abstract

This thesis defines mortality in acromegaly in a modern patient cohort, elucidates underlying explanations for the increased mortality and explores the impact of treatment, focussing on somatostatin analogue therapy. Results confirm there remains a 30% increase in mortality in patients with acromegaly. Mortality was increased in patients with GH >2μg/L, but not in patients with raised IGF-I. This is the first study showing reduced survival in patients with acromegaly following pituitary radiotherapy. Somatostatin analogue therapy was shown to be efficacious and safe.

I also explored factors influencing pituitary tumourigenesis by characterising mRNA levels for 11β-HSD isozymes in normal and neoplastic pituitary tissue. Results demonstrated reduced 11β-HSDl expression and 10-fold increased 11β-HSD2 expression in pituitary tumours compared with normal pituitary, resulting in reduced active glucocorticoid concentrations within the pituitary. This may diminish the antiproliferative effects of glucocorticoids, thus contributing to the process of pituitary tumourigenesis.

Finally, I explored complications of pituitary adenomas by evaluating outcome in patients presenting acutely with pituitary apoplexy. Patients presenting without visual deficit or showing evidence of early improvement in visual deficit can be managed without acute neurosurgical intervention.

Results of this research will undoubtedly improve the management and outcome of patients with acromegaly and pituitary tumours.

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1. INTRODUCTION

1.1 Historical perspective

The word pituitary is derived from the Greek word *ptuo* meaning to spit and the Latin word *pituita* meaning mucous. This is in reference to the fact that when the pituitary gland was first described around 150 AD, its proposed function was to guide waste products from the brain down ducts through the sphenoid and ethmoid bones to the nasopharynx, where they emerged as *pituita* or nasal mucus. It wasn't till 1500 years later that the existence of any communication between the ventricles of the brain and the nasopharynx was disproved (1).

Although the first description of a pituitary adenoma is thought to date from the 17th century (2), it was not till 1886 that Marie proposed a link between such a lesion and a clinical syndrome, acromegaly (3). The importance of the pituitary gland was revealed when Nicolas Paulesco demonstrated that experimental dogs died within 3 days following hypophysectomy (4). He concluded that the pituitary was essential to life, its absence being rapidly fatal. However, it was Harvey Cushing, in his lecture to the American Medical Association in 1909, who introduced the concepts of 'hyperpituitarism' and 'hypopituitarism' with reference to gigantism resulting from overstimulation of growth but understimulation of sexual maturation. He suggested pituitary tumours might result in hypersecretion syndromes whilst causing undersecretion of other substances due to atrophy of the normal gland (5). His theory was confirmed with the identification of further clinical syndromes associated with pituitary neoplasms, including Cushing's disease and hypopituitarism.

The next major advances came with the recognition of the pituitary gland as "the conductor of the endocrine orchestra" and the chemical isolation and bioassay of a number of pituitary hormones. The development of radioimmunoassay technology further revolutionised the measurement of endocrine function, followed by identification and isolation of hypothalamic hormones. Improved techniques in pathology and histology also allowed the identification and classification of the cell types present in normal and adenomatous pituitary tissue.

1.2 Pituitary adenomas

1.2.1 Background

Pituitary adenomas are a diverse group of tumours arising from the pituitary gland. Although the first description of a pituitary adenoma is thought to date from the 17th century, it was Marie who first recognised the link to a clinical syndrome in 1886, a concept which was further elaborated on by Cushing in the early 20th century (1). Historically, these tumours have been classified according to size (microadenomas <1cm and macroadenomas >1 cm in diameter). However, in recent years, this classification has been augmented by a more comprehensive system based on tumour extension, hormonal activity, immunohistochemistry and electron microscopy (6). Pituitary adenomas are predominantly benign lesions, either remaining within the confines of the sella or exhibiting expansive growth within well-demarcated borders. Invasive adenomas may infiltrate dura and bone, but these are not considered malignant as such. The diagnosis of pituitary carcinoma requires the demonstration of cerebrospinal and/or systemic metastases, and not simply the presence of local pituitary tumour invasion (7).

A recent metaanalysis found an overall estimated prevalence of pituitary adenomas of 16.7% (8). The estimated prevalence across postmortem studies was 14.4% compared with 22.5% in radiography studies.

Most pituitary adenomas are monoclonal in origin, suggesting the initiating event in pituitary tumourigenesis occurs within a single abnormal cell which then undergoes clonal expansion. Although several genes have been proposed as playing a role in pituitary tumourigenesis and tumour invasiveness, the underlying pathogenic mechanisms remain poorly understood. The proposed initial event is a genetic alteration in cell proliferation or survival which results in the cells becoming more responsive to hormones or growth factors and promotes clonal expansion (9).

1.2.2 Clinical features

Pituitary adenomas manifest clinically in a number of ways; firstly, tumours which expand beyond the confines of the sella can produce symptoms due to local compressive effects on surrounding structures, including headaches, cranial nerve palsies and visual field defects. Hypersecretion of hormones can cause syndromes such as acromegaly or Cushing's disease. In addition, atrophy or destruction of the remaining normal gland can result in hormone hyposecretion.

Prolactinomas are the most common type of pituitary adenomas, accounting for over 40% of tumours in immunohistochemical studies (8). Hyperprolactinaemia results in amenorrhoea, galactorrhoea and infertility. Other clinical syndromes caused by pituitary adenomas include acromegaly, caused by growth hormone (GH)-secreting pituitary adenomas, and Cushing's

disease, resulting from hypersecretion of adrenocorticotrophic hormone (ACTH). Thyroid stimulating hormone (TSH)-secreting pituitary adenomas are rare, accounting for less than 1% of all pituitary adenomas. The clinical manifestations include hyperthyroidism with inappropriately high levels of TSH for the peripheral thyroid hormone levels. Gonadotroph adenomas are the second most common pituitary adenomas based on immunohistochemical staining, but clinically most of these tumours appear to be non-functioning, even though frequently they produce intact gonadotrophins or their glycoprotein subunits *in vivo* or *in vitro* (10). Symptoms related to hormonal excess are rare and, as with "true" non-functioning adenomas, presentation is often incidental or related to the mechanical effects of a large tumour mass.

Magnetic resonance imaging (MRI) is considered the gold standard for evaluation of the sellar and parasellar regions, as it offers detailed information on pituitary morphology and surrounding structures. The standard protocol for evaluation of the pituitary gland with MRI consists of obtaining T1-weighted images before and after administration of a Gadolinium-based intravenous contrast agent (11).

1.2.3 Management of pituitary adenomas

Management of pituitary adenomas should be aimed at reducing tumour size, normalisation of hormonal excess if present, recovery of normal pituitary tissue and function, and replacement of hormonal deficiencies (12). For prolactinomas, medical therapy with dopamine agonists is very effective in reducing prolactin levels as well as in decreasing tumour size and should constitute the primary mode of treatment (13).

Transsphenoidal surgery is the primary treatment modality for other types of pituitary adenomas, although there is a body of evidence supporting the use of somatostatin analogues for primary medical therapy in a subset of patients with acromegaly (14). Factors influencing surgical outcome include the surgeon's expertise and the size and extension of the adenoma, with extension into the cavernous sinus almost always associated with incomplete surgical removal (15). Postoperative radiotherapy is an option for treatment of residual tumour or persistent hormonal hypersecretion. However, in view of the risks associated with pituitary radiotherapy, mainly hypopituitarism and cerebrovascular morbidity and mortality, the decision to treat with adjuvant radiotherapy must be based on a careful assessment of the balance of benefit and risks in individual patients (16).

Where non-functioning adenomas are not causing compressive symptoms, careful observation and monitoring as opposed to surgical resection is a reasonable option, especially in small intrasellar adenomas. In such cases, periodic monitoring of tumour size using imaging studies is recommended, as up to 20% of these lesions either increase in size or lead to complications such as pituitary apoplexy (12). In all cases, anterior pituitary hormone deficiencies should be appropriately evaluated and treated.

1.2.4 Pituitary apoplexy

Pituitary apoplexy can present as a complication of pituitary adenomas. It presents as a constellation of acute clinical features that include headache, visual deficits, ophthalmoplegia, and alteration in mental status resulting from sudden haemorrhage or infarction of a pituitary adenoma. Pituitary apoplexy as a clinical entity has been recognised for over 100 years (17)

although it occurs only infrequently as a complication in patients with (usually large) pituitary adenomas (0.6 to 9.1% of cases (18-24)).

The pathophysiology of pituitary apoplexy is poorly understood, although a rise in intra-hypophysial pressure as a result of pituitary tumour expansion is likely to alter regional blood flow within the pituitary, disrupting vascular integrity due to hypoxia (25). Taken to its extreme this may result in haemorrhagic infarction of the pituitary.

Certain aspects of the management of pituitary apoplexy remain controversial, with some favouring 'routine' early neurosurgical decompression (22-24), while others advocate a more conservative approach, especially in the absence of significant or progressive neuro-ophthalmological compromise (26,27).

1.3 Acromegaly

1.3.1 Background

The term acromegaly is derived from the Greek words *akron*, meaning extremity, and *megas* meaning great. Acromegaly is a chronic endocrine disease first described by the French neurologist Pierre Marie in 1886. It is caused almost invariably by a GH-secreting pituitary adenoma, although rarely it may be due to a hypothalamic tumour secreting GHRH or ectopic GHRH secretion from a carcinoid tumour (predominantly of the pancreas or bronchus). It is a rare condition, with an estimated prevalence of around 60 per million and an annual incidence of 3-4 per million (28), but active acromegaly is associated with significant morbidity and an increase in mortality compared to the general population (29-32).

1.3.2 Clinical features

The clinical features of acromegaly are due to the somatic and metabolic effects of prolonged excess GH exposure or to local effects of an expanding pituitary mass (33). They often develop insidiously over many years, resulting in delayed diagnosis (34). Most patients experience headaches and sweating. The most typical clinical signs are the coarse facial features, large, spade-shaped hands and enlarged feet resulting from soft tissue swelling and bony enlargement. The facial features include deep nasolabial furrows, prominent supraorbital ridges and enlargement of the lips and nose. Growth of the mandible results in prognathism, malocclusion and widened inter-dental spaces. Other common features include enlargement of the tongue (macroglossia), swelling of the nasopharyngeal tissue, sleep apnoea, lethargy, skin tags, goitre and colonic polyps. The expanding pituitary mass may cause hypopituitarism, reproductive disorders and visual symptoms. GH hypersecretion occurring before the epiphyses have fused results in excess linear bone growth and gigantism.

1.3.3 Complications of acromegaly

The most significant cause of functional disability in acromegaly is arthropathy. Acromegalic arthropathy affects up to 70% of patients and involves both the axial and peripheral skeleton (35,36). Radiological findings include narrowing of the joint spaces, osteophytes and other features seen in osteoarthritis. Symptomatic carpal tunnel syndrome is also a frequent finding, affecting up to 60% of patients, and is thought to be due to oedema of the median nerve in the carpal tunnel, rather than extrinsic nerve compression (35,37).

Acromegaly is characterised by a high incidence of cardiovascular disease, which contributes significantly to morbidity and mortality. Hypertension occurs in around a third of all patients, ranging in some series up to 60% (38). Acromegalic cardiomyopathy is caused by the effects of chronic GH excess on the heart. It is characterised by biventricular concentric hypertrophy, with thickened ventricle walls but normal sized chambers (35). The cardiac hypertrophy is associated with functional alterations (decreased ejection fraction in response to exercise), valve abnormalities and a high incidence of arrhythmias (39-42).

GH counteracts the effects of insulin on glucose and lipid metabolism, resulting in metabolic complications in patients with acromegaly. The most frequent of these is altered glucose metabolism. The prevalence of overt diabetes mellitus ranges from 19 to 56% in patients with active acromegaly, with impaired glucose tolerance (IGT) occurring in up to 46% (35,43). Studies investigating the pathogenesis of altered glucose metabolism in acromegaly suggest GH excess induces insulin resistance by impairing the ability of insulin to suppress gluconeogenesis, decreasing peripheral glucose utilisation, and reducing insulin receptor numbers and binding affinity (35).

The association between the GH/IGF-I axis and neoplasia such as breast, prostate and colon cancer has been the subject of basic and clinical research for many years. However, epidemiological studies exploring the link between acromegaly, cancer incidence and mortality have given rise to conflicting data, leading to significant debate. Early studies suggested an increased incidence of neoplasia overall, particularly of the breast (34) and colon (44), in patients with acromegaly. Evidence from more recent studies, however, has failed to confirm these findings, and suggests overall cancer incidence is not increased in acromegaly (45,46). What has emerged is that patients with acromegaly have an increased risk of developing colorectal cancer,

although the exact magnitude of this risk and the role of screening programmes remain the subject of much debate (47-50). In addition, recent epidemiological studies found cancer death rates in cohorts of patients with acromegaly were similar to those in the general population, suggesting malignancy is not a significant cause of mortality in patients with acromegaly (51,52).

1.3.4 Diagnosis and management

As acromegaly often develops insidiously, few patients present immediately at the time of onset of symptoms and the most common mode of presentation is as an incidental finding by a healthcare professional (53). Biochemical confirmation relies on the demonstration of excessive GH secretion and elevated age- and gender-matched IGF-I.

Magnetic resonance imaging (MRI) is considered the gold standard for investigating pituitary adenomas, providing invaluable information about tumour size and extension, and allowing accurate delineation of parasellar structures (54,55).

The main aims of treatment of acromegaly are reversing the symptoms and signs of the disease, treating the underlying cause, preventing disease recurrence, and improving long-term survival. This involves the use of surgery, radiotherapy and/or medical therapy. Transsphenoidal surgery (TSS) is still considered first line treatment for acromegaly in most patients. Based on strict biochemical criteria (mean GH less than 2.5µg/L [5mU/L], suppressed GH during an OGTT and/or normal IGF-I), the overall remission rate following TSS ranges from 55 to 70% (32,56-60). A number of factors have emerged as crucial in determining outcome following TSS, including tumour size, extrasellar extension, dural invasion and pre-treatment GH levels. Remission rates for microadenomas are around 80 to 90%, while those for macroadenomas are

around 50% (32,56,57,60). The expertise of the pituitary surgeon also plays a key role in determining outcome, as has been illustrated by data from our centre in Birmingham; surgical outcome improved significantly when surgery was performed by a single dedicated pituitary surgeon rather than 8 different surgeons, as had been the case previously (57).

Radiotherapy has been used in the management of acromegaly for nearly a century, with conventional fractionated radiotherapy lowering GH levels over 20 years to less than 5µg/L (10mU/L) in 70 to 90 percent of patients (61). Radiotherapy is also effective at controlling tumour growth as demonstrated by Brada *et al*., with progression-free survival of 94% at 10 years and 88% at 20 years in patients with pituitary adenomas treated with external beam radiotherapy (62). However, a number of factors have led to a re-evaluation of the role of radiotherapy in the management of acromegaly, including the long lag time to clinical effect, formation of secondary intracranial tumours, cognitive impairment, hypopituitarism and cerebrovascular disease.

Traditionally, transsphenoidal surgery and/or radiotherapy have been considered first line treatment for acromegaly, but medical therapy is now used in a significant proportion of patients. Current options include dopamine agonists, somatostatin analogues and more recently GH receptor antagonists.

The dopamine agonist bromocriptine was the first effective medical treatment for acromegaly, but it lowers GH secretion to the desired levels in less than 20% of cases and is associated with a number of side effects including nausea, dizziness and headaches (63). Newer dopamine agonists like cabergoline are better tolerated and more efficacious, lowering GH levels to less than 2µg/L (4mU/L) in up to 46% of cases, with greater responses in patients with tumours co-secreting GH and prolactin (64). This is not uncommon, as up to one third of these adenomas are plurihormonal acidophil cell-derived tumours and co-secrete both hormones (65).

The use of dopamine agonists has largely been superseded by the introduction of somatostatin analogues which exert their biological effects by activating somatostatin receptors (predominantly sub-receptor types 2 and 5) in the pituitary (66). Octreotide, a long-acting synthetic somatostatin analogue, has been used to treat acromegaly for 2 decades, and since the mid-1990s, three slow-release depot preparations have been introduced; Sandostatin LAR (Novartis), Lanreotide LA (Ipsen) and Lanreotide Autogel (Ipsen). These have been shown to be both effective and safe, suppressing GH levels to less than 2-2.5µg/L (4-5mU/L) and normalising serum IGF-I levels in 50-70% of cases (66-69). In addition, tumour shrinkage by 20-50% has been documented in around 30% of patients pre-selected for octreotide responsiveness (66,70-73).

Although somatostatin analogues have traditionally been used as an adjunct to surgery and radiotherapy, they are increasingly being used as first line therapy in the treatment of acromegaly. Several studies in which somatostatin analogues were administered to previously untreated (*de novo*) patients demonstrated suppression of GH and IGF-I to a similar extent to that observed in patients who received the drugs after surgery (14,71,74,75). These observations have led the authors to conclude that if the possibility of surgical cure is low, and if there is no visual compromise, then medical treatment with somatostatin analogues alone is as effective biochemically and clinically as the combination of surgery followed by medical therapy, and offers a reasonable primary therapeutic modality.

Given the recognised efficacy of somatostatin analogues in improving biochemical parameters and reducing tumour size, a number of studies have investigated the impact of pre-treatment with these drugs on surgical outcome. Whilst some have demonstrated no clear benefits, others have

found improvements in terms of remission rates and clinical condition, including preoperative blood pressure, cardiac function, glucose metabolism and shorter hospital stays (76,77).

There are a number of side effects associated with somatostatin analogue therapy, but these are rarely severe and in general do not limit therapy. Around 50% of patients experience gastrointestinal symptoms including diarrhoea, nausea and abdominal discomfort, but these are usually transient (66). New gallstone formation (usually asymptomatic) occurs in 10-20% of patients and a small number develop impaired glucose metabolism (14).

Pegvisomant is a novel, genetically engineered GH receptor antagonist that, in contrast to dopamine agonists and somatostatin analogues, inhibits GH action rather than secretion. It exerts its biological actions by preventing functional dimerisation of the GH receptor (78). Clinical studies have demonstrated that pegvisomant is remarkably effective, improving clinical symptoms and signs and resulting in IGF-I normalisation in over 90% of patients (79-81). The drug appears to be safe and well tolerated. Because pegvisomant works by blocking the actions of GH, efficacy is independent of tumour characteristics, such as the density of somatostatin receptors. Despite normalised IGF-I levels, GH levels remain elevated in these patients, albeit with minimal or neutralised bioactivity because of receptor blockade. Concerns surrounding tumour growth, deranged liver function and the clinical impact of antibody formation are being addressed as experience with the use of these drugs grows (82).

1.4 Aims of project

Using acromegaly as a model of pituitary disease, the aims of this project are;

1. To define mortality in acromegaly in a modern cohort of patients using data from the West Midlands Acromegaly Database
2. To elucidate the underlying explanation for the increased mortality in acromegaly by examining both hormonal and non-hormonal factors and their association to mortality
3. To carry out long-term surveillance on patients with acromegaly treated with somatostatin analogues, evaluating outcomes in these patients including efficacy and safety profiles, as well as tachyphylaxis, tolerability and side effects. In this section I will also examine factors influencing the decision to treat patients with acromegaly with primary medical therapy and evaluate outcome in these patients.
4. To explore factors influencing pituitary tumourigenesis by characterising mRNA levels for 11β-HSD1 and 2 in a large cohort of normal and neoplastic pituitary tissue, including tissue from a subgroup of patients with acromegaly.
5. To explore complications of pituitary adenomas by evaluating clinical presentation, management, and clinical outcomes in a cohort of patients (including a small number with GH-secreting adenomas) who presented acutely with pituitary apoplexy.

2. DEFINING MORTALITY IN ACROMEGALY

2.1 Introduction

Although it had not been convincingly investigated and reported, patients with acromegaly were thought to have reduced life expectancy as far back as the 1920s. In a series of 100 cases of acromegaly studied and reported on in 1966, 50% had died before the age of 50 years and 89% by the age of 60 years (83). The causes of death in these early series were diverse, ranging from diabetic coma and vascular disease to sepsis and extension of the pituitary tumour.

The excess mortality associated with acromegaly was first accurately qualified and quantified in the series by Wright *et al*., published in 1970 (29). The ages at death and causes of death in a cohort of 194 subjects with acromegaly were analysed and compared with that of the general population of England and Wales. 54 deaths were observed, compared with 28.5 expected, giving a standardised mortality ratio (SMR) of 1.9. The increased number of deaths was due to cardiovascular disease in males, cerebrovascular disease in females and respiratory disease in both. There was no increase in deaths from malignancies. Factors associated with increased mortality included the presence of hypertension and diabetes mellitus. Even in this early study, mortality rates were found to be lower in those patients who had received treatment for their acromegaly compared with those who had not received any treatment.

Several retrospective studies have since confirmed these findings, demonstrating a 2- to 3- fold increased mortality in patients with acromegaly compared with age- and sex-matched controls, with death predominantly due to vascular disease, respiratory disease and, in the earlier studies, malignancy (30,31,34,45,51,60,84). However, results from the more recent studies also

demonstrated that the increased mortality associated with acromegaly can be diminished if treatment is successful in reducing GH hypersecretion to less than 2-2.5μg/L (4-5mU/L), whether this is measured as the mean of a growth hormone day profile or as a random growth hormone level (30,31,45,51,52).

In this project, data from the West Midlands Acromegaly Database were used to determine whether the poor long-term outcome associated with acromegaly has improved with the advent of more effective treatment strategies, most notably the introduction of somatostatin analogues.

2.2 Patients

The West Midlands Acromegaly Database was established in 1990 and on 31st December 2001 contained demographic and clinical details of 419 patients (241 female) with acromegaly from 16 referral centres across the West Midlands Region (Figure 2.1). The region has an overall population of 5.7 million. All patients had a firm biochemical diagnosis of acromegaly, based on currently accepted criteria (failure of GH suppression to less than 1 μg/L after oral glucose loading and in most cases an elevated IGF-I). All patients with samples showing elevated GH or IGF-I in the West Midlands Regional Endocrine Laboratory were flagged as potentially having acromegaly and appropriately assessed; therefore patient capture was good and there are no grounds to assume that selection bias is substantial. However, a small number of patients had died before the measurement of serum IGF-I came into routine clinical use across the region in the early 1990's. 136 patients were treated with surgery alone, 91 with radiotherapy alone (total dose 45 to 50 Gy in 30 treatments via three ports) and 120 with both surgery and radiotherapy. 71

patients were treated with primary medical therapy alone, having received no definitive treatment (surgery or radiotherapy) previously.

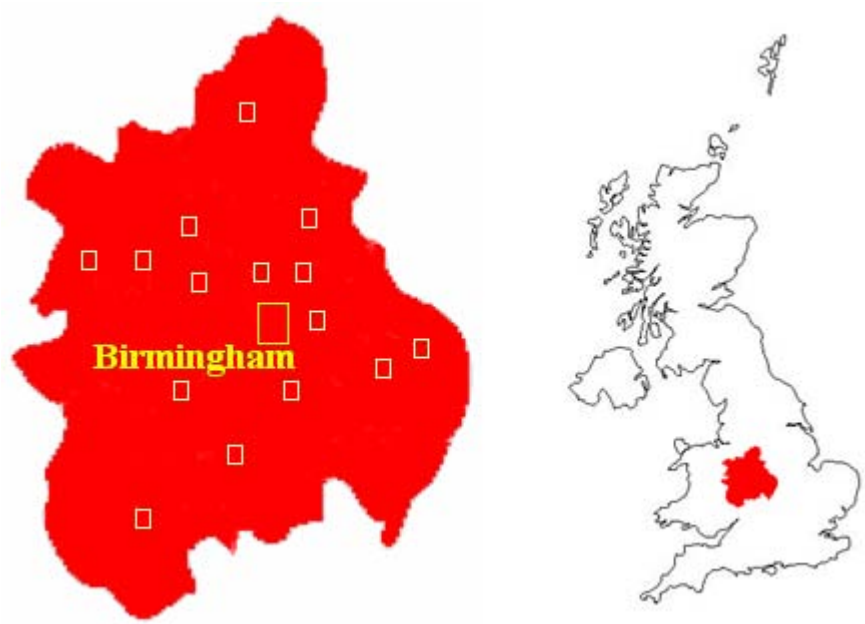
The study was approved by the local research ethics committee of each site and the Office of National Statistics (ONS). All patients were registered with the Office of National Statistics and death certification data from ONS were reviewed to obtain information relating to cause of death according to ICD-9 criteria.

324 patients were alive on the exit date of the study (31st December 2001) and 95 deceased. Median age at diagnosis was 47 years (range 12-84) in the entire cohort, 44 years (12-73) in those still alive and 53 years (29-84) in those who had died. Median duration of follow up was 13 years (0.5-48), which was similar in both the deceased and alive groups.

2.3 Endocrine evaluation

Prior to December 2000, serum GH was measured using an in-house RIA calibrated against IS 80/505 at the Regional Endocrine Laboratory at the University Hospital Birmingham, Selly Oak, as previously described (85). The standard (IS 80/505) was assigned a value in International Units, but never given an official equivalent value in mass units and therefore GH was reported in mIU/L. The value in mIU/L was divided by a conversion factor of 2 to obtain μg/L. Limit of detection of the assay was 0.5 μg/L, whilst intra-assay coefficient of variation (CV) was 4.1 % and inter-assay CV 5.7 % at 2 μg/L. From December 18th 2000, the assay changed to the DPC Immulite 1000 immunometric assay calibrated against IS 80/505. For results < 20 mIU/L the DPC assay gave similar results to the RIA. For results >20 mIU/L the DPC assay gave results between 10-20% lower.

Figure 2.1



Centres referring patients to the West Midlands Acromegaly Database: Queen Elizabeth and Selly Oak Hospitals, Birmingham; County Hospital, Hereford; Birmingham Heartlands Hospital; Staffordshire District General Hospital; City Hospital, Birmingham; Coventry and Warwick Hospital; North Staffordshire Hospital; Worcester Royal Infirmary; New Cross Hospital, Wolverhampton; Alexandra Hospital, Redditch; Burton District Hospital; Sandwell Hospital; Telford District General Hospital; Manor Hospital, Walsall; Wordesley Hospital.

The assay was transferred to the Immulite 2500 when DPC was bought by Siemens. This did not result in any significant change in GH values.

A new international standard was produced in 2001 (WHO IS 98/574). This is comprised of recombinant material consisting of a 22 kDa growth hormone of >95% purity (86). Unlike the previous standard, IS 98/574 has been assigned values in both mass and International Units, allowing conversion between mass units and International Units such that 1μg corresponds to 3 milli-International Units. Although our GH assay has remained the same (Siemens Immulite 2500), it has now been calibrated against the new IS 98/574 and since May 12th 2008, results have been reported in μg/L.

Assessment of GH secretion following treatment differed between units. Levels were recorded as the mean of 5 GH measurements across a 2-hour 75 g oral glucose tolerance test (OGTT) (n=96), the mean of a GH day profile (the average of 5 GH measurements taken at 2 hour intervals) (n=50) or a random GH performed in an outpatient setting (n=268). Data on GH levels were available in all but 5 patients. The lowest GH achieved was considered to be < 2 μg/L if either the mean of 5 GH measurements across a 2-hour 75 g oral glucose tolerance test or the mean of a GH day profile was below this limit or if at least 2 random GH measurements were < 2 μg/L.

Serum IGF-I was measured using an in-house RIA calibrated against IRR 87/518 with acid-ethanol extraction performed to remove IGF binding proteins, as previously described (67). The limit of detection of the assay was 2.0 nmol/L. Inter-assay CV was 5.4 - 8.4% between 16-104 nmol/L. Reference ranges were derived from adults with no known or suspected endocrine disorders. Results were calculated as the mean ± 2 SD and expressed by age. Values were 14-48 nmol/L at 21-30 y (N= 71), 13-37 nmol/L at 31-45y (N= 123) and 8.9-32 nmol/L at >45y (N= 75). IGF-I data were available in 360 of the 419 patients.

2.4 Statistical analysis

Statistical analysis was performed by Dr Michael Hills, Senior Lecturer (retired), Department of Medical Statistics, London School of Hygiene and Tropical Medicine. An external comparison of the entire cohort with the general population was made using the Standardised Mortality Ratio (SMR) based on published mortality data for England and Wales by 5-year age and calendar periods. Confidence intervals and p-values were obtained using the normal approximation in which the standard error of the natural logarithm of the SMR is 1 divided by the square root of the observed number of deaths for the confidence interval and the expected number of deaths for the p-value.

2.5 Results

All cause mortality was significantly increased compared to the general population (SMR = 95/75.5 = 1.26, [1.03-1.54]; p=0.046). The causes of death are outlined in Table 2.1. The excess mortality was due mainly to cerebrovascular disease (SMR = 20/7.5 = 2.68, [1.73- 4.15]; p=0.007), with small but non-significant increases due to cardiovascular and respiratory disease. There was no increase in deaths due to malignancy.

Cause	Observed Deaths	Expected Deaths	SMR (95% CI)	p
All cause	95	75.5	1.26 (1.03-1.54)	0.045
Cerebrovascular	20	7.5	2.68 (1.73-4.15)	0.007
Cardiovascular	35	25.6	1.37 (0.98-1.90)	0.111
Respiratory	13	8.6	1.52 (0.88-2.61)	0.219
Malignancy	21	23.1	0.91 (0.59-1.39)	0.650

Table 2.1: All cause and cause specific mortality in acromegaly

2.6 Discussion

Several retrospective epidemiological studies have demonstrated a 2- to 3-fold increase in mortality in patients with acromegaly compared with age- and sex-matched control populations (29-32,34,45,60,84). This has been reported to equate to an average reduction in life expectancy of ten years (31). Whilst there is still a 30% increase in mortality in this study, survival does appear to be improving when compared to earlier studies. However, the median age at death is lower than in previous studies, which may reflect the age and sex distribution of our cohort.

In recent years, significant advances have been made in the management of acromegaly. This is reflected in the change in overall mortality rates seen in modern cohorts of patients with acromegaly. In epidemiological studies performed over the last decade (32,45,51,60,87-89), although mortality in patients with acromegaly remains elevated compared to the general population, the mortality increase is generally less than 2-fold, compared with the 2- to 3-fold

mortality rates seen in earlier series (Tables 2.2 and 2.3). This may in part be due to improved multidisciplinary care and a greater awareness of the benefits of reducing GH hypersecretion. In a recent meta-analysis, SMR of greater than 1 was reported in all 16 studies included (90). The reported SMRs ranged from 1.16 to 3.31, with a mean weighted SMR of 1.72. A meta-regression pointed towards improved survival in more recent studies, presumably due to modern treatment modalities and more strictly defined cure criteria.

The predominant cause of death in this cohort was vascular disease, supporting the findings of previous studies. Holdaway *et al*. examined mortality data in 208 patients with acromegaly, comparing outcome with the general population of New Zealand (51). During the period of follow-up, 72 patients died (35% of the total group). The proportion of patients dying from neoplastic disease and stroke was broadly similar to the values expected for the general New Zealand population, but cardiovascular deaths were increased compared with expected values. When assessed by multivariate analysis, last serum GH ($P < 0.001$), age, duration of symptoms before diagnosis ($P < 0.03$), and hypertension ($P < 0.04$) were independent predictors of mortality. As mortality from cardiovascular disease has been found to be increased in acromegaly in several studies (34,51,84,91), it is somewhat surprising the only cardiac risk factor identified in multivariate analysis that might directly impact on mortality was hypertension. However, it is likely that low patient numbers or incomplete data have underemphasised a potential influence of other classical cardiovascular risk factors on mortality, such as diabetes, smoking and dyslipidaemia (96). This apparent lack of impact of traditional cardiac risk factors, in the face of elevated cardiovascular mortality in acromegaly, suggests a role for the specific deleterious effect of increased serum levels of GH and IGF-I on cardiac function as detailed by Colao *et al*. (35).

Reference	Patients	Deaths	Mortality Cause
Wright et al, 1970	194	55	Total Group SMR 1.8 Cause specific Vascular 38.5% Respiratory 18% Malignant 18%
Alexander et al, 1980	164	45	Total Group SMR 3.3 (Male 24/5, SMR=4.8, Female 21/8.1, SMR=2.6) Cause Specific Vascular 60% Respiratory 15.5% Malignant 15.5%
Nabarro 1987	256	47	Total Group SMR1.3 (< 55 years 10/5.3, SMR=1.9, Female 23/13.7, SMR=1.7) Cause specific Cardio/cerebrovascular 47/37.2 SMR = 1.3 N/S Vascular 55% Respiratory 6% Malignant 23%
Bengtsson et al, 1988	166	62	Total Group SMR 3.2 Cause specific Vascular deaths 32/9 SMR=3.6 Cancer deaths 15/5.6 SMR=2.7
Rajasoorya et al, 1994	151	32	Total Group SMR 3.0 Cause Specific Cardiovascular SMR 3 Cerebrovascular SMR 3 Malignancy SMR 1
Extabe et al, 1993	74	10	Total Group SMR 3.2 (1.55-5.93) [Male SMR 7 (2.81-14.4), Female SMR 1.4 (0.29-4.17)] Cause specific Vascular 10 (0.25-55.7) Malignancy 7.1 (2.31-16.6)
Bates et al 1993	79	28	Total Group SMR 2.63 (1.8-3.9) Cause specific Vascular 57% Respiratory 25% Malignant 11%
Orme et al 1998	1362	366	Total Group SMR 1.60 (1.44-1.77) Cause specific Vascular SMR 1.76 (1.47-2.07), p<0.001 Cerebrovascular SMR 2.06 (1.5-2.76), p<0.001 Respiratory SMR 1.85 (1.34-2.49), p<0.001 Malignant SMR 1.16 (0.92-1.44), p=0.1
Swearingen et al 1998	149	12	Total Group SMR 1.16 (0.66-2.0) Cause specific Vascular 5/12 Respiratory 1/12 Malignant 4/12
Abosch et al 1998	254	29	Total Group SMR 1.28 Cause specific not available in majority of 20 deaths

Table 2.2: Studies assessing disease specific mortality rates in patients with acromegaly pre year 2000 [References (29-32,34,45,84,91-93)]

Reference	Patients	Deaths	Mortality Cause
Beauregard et al 2003	103	18	Total Group SMR 2.14 Cause specific Vascular 5/18 Malignant 9/18
Arita et al 2003	154	11	Total Group SMR 1.17 (0.54-2.38) Cause specific Vascular 4/11 Respiratory 2/11 Malignant 2/11
Biermasz et al 2004	164	28	Total Group SMR 1.33 (0.87, 1.87) Cause specific Vascular 7/28 Malignant 13/28
Holdaway et al 2004	208	72	Total Group SMR 1.22 Cause specific Vascular 36/72 (50%) Respiratory 2/76 Malignant 17/72 (24%)
Mestron et al 2004	1219	56	Total Group SMR not available SMR 1.3 (0.52-2.67) for remission group and 1.38 (0.51-3.0) in persistent disease group Cause specific Cardiovascular 26.8% Cerebrovascular 8.9% Respiratory 5.4% Malignant 16.1%
Kauppinen-Makelin et al 2005	334	56	Total Group SMR 1.16 (0.85-1.54) Cause specific Cardiovascular 23.2% (coronary artery disease) Other cardiovascular diseases (16.1%) Cerebrovascular 14.3% Malignant 21.4%
Trepp et al 2005	94	13	Total Group SMR 1.34 (0.71-2.29) Cause specific Cardiovascular 6/13 Malignant 4/13
Sherlock et al 2009	501	162	Total Group SMR 1.7 (1.4-2.0), p<0.001 Cause specific Cardiovascular SMR 1.9 (1.6-2.4), p>0.001 Cerebrovascular SMR 2.7 (1.9-4.1), p<0.001 Respiratory SMR 1.8 (1.1-2.8), p=0.01 Malignant SMR 1.2 (0.9, 1.7), p=0.26

Table 2.3: Studies assessing disease specific mortality rates in patients with acromegaly post year 2000 [References (51,52,60,88,89,94,95), Sherlock et al. unpublished data]

In our study, as in others (29,34,84), an increased death rate in acromegaly from cerebrovascular disease has also been identified, supporting a role for the influence of hypertension and other classical vascular risk factors on mortality from stroke in these patients. Other factors which may contribute to cerebrovascular morbidity and mortality are microvascular abnormalities specific to elevated circulating GH levels. Nailfold capillaroscopy *in vivo* has demonstrated significantly reduced numbers of capillaries per mm, shorter length of capillaries, but increased numbers of tortuous loops and meandering capillaries in patients with acromegaly (97). More recently, using pressure myography to examine subcutaneous blood vessels from gluteal fat biopsies harvested from 18 patients with active acromegaly, Paisley *et al*. demonstrated increased wall thickness, wall:lumen ratio and growth index as well as endothelial-dependent dysfunction compared to controls (98).

Some early studies suggested an increased incidence of neoplasia overall, particularly of the breast (34) and colon (44), in patients with acromegaly. Evidence from more recent studies, however, has failed to confirm these findings, and suggests overall cancer incidence is not increased in acromegaly (45,46). Recently a number of publications have described the associations between GH, IGF-I and cancer in both animal models and humans. These include altered risk of cancer associated with polymorphisms in genes within the GH/IGF-I axis (99-101) and increased GH expression in human proliferative disorders (102,103). The role of IGF-I and the IGF-I receptor in oncogenesis is well documented. Elevated serum levels of IGF-I have been associated with an increased risk of breast, prostate and colorectal cancer in humans (104-106). *In vitro* data, animal studies, and epidemiological studies in non-acromegalic patients have implicated the GH/IGF-I axis in the development of breast, prostate and thyroid cancer (107). IGF-I causes marked proliferation of breast cancer cells which can be inhibited by an anti-IGF-I

receptor antibody (108,109). In addition, transgenic mice over-expressing human GH (110) or IGF-I (111) display an increased incidence of mammary tumours. Conversely, *lit/lit* mice with a defective GH-releasing hormone receptor and thus decreased GH/IGF-I levels show reduced growth of transplanted human breast cancer cells (112). Furthermore, epidemiological studies in non-acromegalic patients with breast cancer have suggested that these patients have significantly higher serum GH and IGF-I levels compared to the general population (113,114) and that high IGF-I levels predict a 4.6-fold higher risk of developing breast cancer in premenopausal women under the age of 50 (115). Similarly, treatment of primary cultures of prostate tumour epithelial cells with IGF-I causes cell proliferation which can be inhibited by IGF-I receptor antisense oligonucleotide transfection (116,117) and transgenic mice expressing human IGF-I in prostrate epithelial cells demonstrate a stepwise development of prostatic adenocarcinomas (118). In addition, retrospective population studies have shown that prostate cancer is associated with IGF-I levels in the higher normal range (119,120) and a prospective study demonstrated that men in the highest quartile of IGF-I levels had a 4-fold increased risk of developing prostate cancer compared with men in the lowest quartile (121).

Much of the literature exploring the issue of cancer risk in acromegaly has focussed on colorectal cancer. The majority of colonic carcinomas are thought to arise from benign adenomatous polyps over a period of 10 to 20 years (122,123). Many studies have therefore attempted to quantify colorectal cancer risk using the prevalence of adenomas found at colonoscopy as a surrogate. The findings of these studies were comprehensively reviewed in a recent article by Renehan and Brennan (124). Most showed increased prevalence of adenomatous polyps, supporting the notion that acromegaly is associated with increased colorectal cancer risk. One prospective study evaluated the prevalence of carcinoma, premalignant adenomas and hyperplastic colonic polyps

in one hundred and twenty-nine patients with biochemically proven acromegaly by colonoscopic examination (125). 5% had an adenocarcinoma and one or more tubulovillous adenoma was found in 34 patients (26%). Compared with controls, the odds ratio of colorectal cancer was increased at 13.5. Renehan and Brennan argue, however, that these studies overestimate the risk of developing colorectal cancer in acromegaly. Reasons for this include the small patient numbers and inability to adjust for major confounding factors such as age and gender, inappropriate control populations, use of unblinded endoscopists and the knowledge that not all adenomas will invariably progress to become carcinomas. For these reasons they believe that a more appropriate method for assessing risk is to use population studies.

Three population studies have attempted to address this issue: the Veteran's study (44), the UK Acromegaly Group study (45), and the Sweden and Denmark population study (126). In the Veteran's study, Ron *et al*. reviewed hospital records of 1041 male patients with acromegaly admitted to the United States Veteran Affairs hospitals and documented 116 cases of cancers (including 13 colon cancers) and an increased tumour incidence compared to tumour incidence rates from 3.7 million first admissions to Veteran Affairs hospitals (standardised incidence ratio (SIR) 1.6, SIR for colon cancer 3.08). Orme *et al*. analysed a large cohort of 1239 patients with acromegaly, representing 16,778 person-years. 79 cancers were observed in the patient group while 104 were expected from calculations utilising data from the national cancer register for England and Wales (SIR 0.76). Sub-analysis of the incidence of different tumour types revealed a non-significant increased incidence of colon cancers in patients with acromegaly (SIR 1.68). Baris *et al*., on the other hand, found an increased risk for all cancers in 1634 patients with acromegaly from Sweden and Denmark compared to the general population (SIR 1.5). Risk was increased for all gastroenteropancreatic (including colorectal), kidney, thyroid, and, in females,

breast cancers, while prostate cancer incidence was not significantly increased. The summary risk ratio for colorectal cancer estimated from these studies is 2.04, suggesting a modestly increased risk.

Thus, despite the observations from the *in vitro* and animal studies discussed above, the large population studies have not demonstrated an increased incidence of breast or prostate cancer in patients with acromegaly (45,126). However, they all reported an increased risk of thyroid cancer compared with the general population (44,45,126). A meta-analysis of these studies yields a summary risk estimate of 3.64 (95% CI: 1.63, 8.11) for thyroid cancer (124).

There are a number of potential biological mechanisms which may explain the pathogenesis of tumour development in acromegaly. These include direct GH/IGF-I actions, hyperinsulinaemia, altered IGFBP-3, increased IGF-I and IGFBP-2 levels, altered local immune responses and shared genetic susceptibility (50,107). Elevated circulating GH levels have direct mitogenic and anti-apoptotic effects in many tissues through transcriptional regulation of different signalling pathways (101). The mitogenic and anti-apoptotic properties of IGF-I may favour tumour formation, invasion and metastasis (127,128). These effects are mediated via the widely-expressed IGF-I receptor.

Glucose intolerance is a common complication in acromegaly, affecting over 50% of patients (35). Several epidemiological cohort studies have indicated a direct association between circulating C-peptide or insulin levels and various cancers; a recent meta-analysis of prospective studies showed excess risks of colorectal and pancreatic cancers associated with higher levels of circulating C-peptide/insulin and with markers of glycaemia (129). In a cohort of patients with acromegaly undergoing screening colonoscopy, Colao *et al*. found significantly lower fasting insulin levels in patients without lesions (16.0 +/- 7.5 mU/L) than in patients with hyperplastic

polyps (22.4 +/- 8.8 mU/L; P < 0.01), adenomatous polyps (38.0 +/- 15.9 mU/L; P < 0.0001) and adenocarcinoma (59.0 +/- 30.6 mU/L; P < 0.0001) (130).

In common with multiple other non-pituitary tumours, epigenetic modification is a feature of pituitary tumourigenesis. A recent study utilising the nationwide Swedish Family-Cancer Database to analyse familial risk for pituitary adenomas and associated tumours found an increased risk of skin cancer (SIR 1.60; CI: 1.13–2.21), leukaemia (SIR 1.90; CI: 1.31–2.66) and nervous system haemangiopericytomas (SIR 182) in parents of patients with pituitary adenomas (131). Among siblings there was a significant association between pituitary tumours and breast cancer (SIR 1.46; CI: 1.02–2.01), while the risk of pituitary adenoma was marginally increased in individuals whose siblings were diagnosed with colorectal cancer (SIR 1.53; CI: 0.69–2.92). A potential candidate gene which could explain these findings is the recently described aryl hydrocarbon receptor interacting protein (AIP) gene, but to prove this hypothesis, germline AIP mutations will need to be examined in the context of additional common somatic changes in the pituitary and other affected organs (107).

Despite the findings of increased cancer incidence in some studies, it is of interest that in 2 studies addressing the issue of cancer mortality in acromegaly (45,51), increased mortality rates for all cancers was only demonstrated in patients with higher post-treatment GH and IGF-I levels, while if post-treatment GH and IGF-I were controlled, cancer mortality was similar to the general population. Orme *et al*. found the overall cancer mortality rate was not increased, but there was a significant increase in the colon cancer mortality rate (SMR 2.47) and a non-significant increase in female breast cancer mortality (SMR 1.60) in the cohort as a whole. However, there was a 1.8-fold increased mortality risk for all cancers, a 4.6-fold increased mortality risk of colon cancer and a 2.9-fold increased mortality risk of breast cancer if serum GH levels were > 10 ng/ml (45).

Thus increased mortality from cancer is only significant if GH levels are uncontrolled. Most other recent epidemiological studies have found cancer death rates in cohorts of patients with acromegaly are similar to those in the general population, suggesting malignancy is not a significant cause of mortality in patients with acromegaly (52,89).

Taken together, these findings suggest patients with acromegaly may have an increased risk of developing colorectal cancer, although the exact magnitude of this risk and the role of screening programmes remain the subject of much debate. Current British Gastroenterology Society guidelines recommend patients with acromegaly should be offered regular colonoscopic screening, starting at the age of 40 years, with the frequency of repeat colonoscopy depending on the findings at the original screening and the activity of the underlying acromegaly (132). Patients with an adenoma at first screening or increased serum IGF-I should be offered screening at three year intervals and those with either a negative first colonoscopy or a hyperplastic polyp should be offered screening at five year intervals. Several groups have argued that such an approach is too intensive and potentially harmful, and suggest that as patients with acromegaly have risks just above those for average-risk individuals, it would be reasonable to offer colonoscopic screening in these patients at the age of 50–55 years (49,133). Surveillance following initial colonoscopic screening should be determined primarily by clinicopathological findings, as is the case in general population screening (124).

In conclusion, I have demonstrated that although mortality in patients with acromegaly remains elevated compared to the general population, the mortality increase is generally less than was seen in earlier series (Table 2.2). This is most likely due to improved multidisciplinary care and a greater awareness of the benefits of reducing GH hypersecretion.

In Chapters 3 and 4, I will discuss the underlying explanation for the increased mortality rates seen in acromegaly.

3. UNDERLYING EXPLANATION FOR INCREASED MORTALITY IN ACROMEGALY- HORMONAL FACTORS

3.1 Introduction

As discussed and demonstrated in Chapter 2, untreated acromegaly is associated with reduced life expectancy. Several retrospective studies have demonstrated a 2- to 3- fold increased mortality in patients with acromegaly compared with age- and sex-matched controls (29-31,34,45,51,60,84). However, results from the more recent studies also demonstrated that the high mortality rates associated with acromegaly can be reversed if treatment is successful in reducing GH levels to less than 2-2.5µg/L (30,31,45,51,52).

As discussed in Chapter 2, in the West Midlands Acromegaly Study I reported on outcome in 419 patients with acromegaly, of whom 324 were alive and 95 deceased. Compared to the general population, all cause mortality was significantly increased with an SMR of 1.26 (1.03-1.54). The excess mortality was due predominantly to cerebrovascular disease with small but non-significant increases due to cardiovascular and respiratory disease (Table 2.1).

In this part of the project, I explored possible underlying causes for the increased mortality in acromegaly by examining hormonal factors which may play a role in this increased mortality. The aim was to explore in greater detail the biochemical targets for therapy. In addition, the development of an effective therapeutic GH antagonist (79,80), where GH cannot be used to monitor treatment, has heightened the need for robust data confirming the utility of IGF-I as a biochemical marker of effective treatment.

I examined outcomes of patients with normal and elevated levels of IGF-I and contrasted them with outcomes of patients with and without persistent GH hypersecretion. Since hypopituitarism is known to confer excess mortality in patients with non-functioning adenomas (134), I also assessed the impact of other pituitary hormone deficiencies on long-term outcome in acromegaly.

3.2 Patients

The West Midlands Acromegaly Database was established in 1990 and on 31st December 2001 contained demographic and clinical details of 419 patients (241 female) with acromegaly from 16 referral centres across the West Midlands Region. Details of the patient cohort are described in detail in Section 2.2.

3.3 Endocrine evaluation

Assay and measurement details are outlined in Section 2.3. Serum GH levels were measured by an in-house RIA at the Regional Endocrine Laboratory at the University Hospital Birmingham, Selly Oak, as previously described (85). Serum IGF-I was measured using an in-house RIA with acid-ethanol extraction performed to remove IGF binding proteins, as previously described (67). IGF-I data were available in 360 of the 419 patients.

The presence or absence of hypopituitarism was defined by proven biochemical deficiency of at least one endocrine axis. The hypothalamo-pitutary-adrenal axis was deficient if the peak cortisol response to short synacthen testing was less than 530 nmol/l (135) or less than 500 nmol/l following an insulin stress test. The thyroid axis was deficient if the free thyroxine concentration

was below the local reference range. A serum testosterone level below the local reference range defined hypothalamic-pituitary-gonadal dysfunction in males. In pre-menopausal females, deficiency was assumed if the serum prolactin was normal and the patient amenorrhoeic and in post-menopausal females if the FSH was inappropriately low (< 35 IU/L).

3.4 Statistical analysis

Statistical analysis was performed by Dr Michael Hills, Senior Lecturer (retired), Department of Medical Statistics, London School of Hygiene and Tropical Medicine. An external comparison of the entire cohort with the general population was made using the Standardised Mortality Ratio based on published mortality data for England and Wales by 5-year age and calendar periods as described in Chapter 2. Internal comparisons between groups were made, using the ratio of mortality rates (RR), obtained with the statistical package Stata (Stata Corp. 2001, Stata Statistical Software: Release 7.0. College Station, TX: Stata Corporation) using Poisson regression to control for age and sex. Multiple exponential regression was used to determine the effect of serum GH and IGF-I.

3.5 Results

3.5.1 Effect of growth hormone reduction on mortality

Previous studies have adopted arbitrary cut off points to define an adequate response to treatment. There has been little scientific basis to the selection of these cut off points. In this study,

comparison of crude death rates per 1000 population suggests that a GH of 2 μg/L is an appropriate target, with a step-up in death rates when this target was exceeded (Table 3.1).

Lowest GH achieved (μg/L)	Number of deaths	Death rate per 1000
0 - 0.5	6	10.5
0.5 - 1.0	13	9.2
1.0 - 1.5	5	6.6
1.5 - 2.0	3	6.7
2.0 - 2.5	11	23.7
2.5 - 5.0	30	26.0
5.0 - 50.0	23	23.3
> 50.0	3	30.8

Table 3.1: Crude death rates in acromegaly related to the lowest GH achieved during follow up

The mortality in 202 patients with GH > 2 μg/L was compared with 216 patients with GH < 2 μg/L. In patients achieving lowest GH < 2 μg/L, SMR was 1.10 (0.76-1.67); p=NS, compared with an SMR of 1.31 (1.03-1.66); p= 0.05, in those with lowest GH > 2 μg/L (Table 3.2).

Group	Observed Deaths	Expected Deaths	SMR (95% CI)	P
Lowest GH < 2 μg/L	27	24.2	1.10 (0.76-1.63)	NS
Lowest GH > 2 μg/L	67	51.2	1.31 (1.03-1.66)	0.05

Table 3.2: Impact of GH reduction on mortality in acromegaly

3.5.2 Impact of age on the long-term effects of GH hypersecretion

Analysis by age suggests that younger patients who fail to achieve a GH target of 2 µg/L are at greater risk than older patients. The rate ratio comparing subjects with GH > 2 µg/L to those with GH < 2 µg/L was 4.46 (0.9-23.0) in age group 40-50 years, falling to 3.40 (1.1-11.1) in the 50-60 year group, 1.69 (0.8-3.60) in the 60-70 group and 0.70 (0.3-1.4) in those aged between 70 and 80 years. The trend for the rate ratio to decrease with increasing age is significant (p=0.01).

3.5.3 Effect of IGF-I normalisation on mortality

IGF-I data were available in 360 patients, representing 86 % of the cohort - 125 were classed as above the age-related normal range and 235 were within the normal range. Of the patients with persistently elevated IGF-I, 36 had a lowest GH of less than 2 µg/L (10 % of the entire cohort), whilst of those with a normal IGF-I, 69 had a lowest GH of more than 2 µg/L (19 % of the entire cohort). Thus, in total there was a discrepancy between GH status and serum IGF-I in 29% of the cohort.

No effect of IGF-I on outcome could be demonstrated. Internal comparison of these groups, controlled for age and sex, revealed a rate ratio of 1.20 (0.71-2.02), p=0.50. However, although numbers are small and uncontrolled for age and sex, comparison of crude death rates in groups with normal or elevated levels of serum IGF-I and serum GH greater or less than 2 µg/L suggests that normalisation of serum IGF-I may have some effect in reducing the mortality associated with elevated serum GH levels (Table 3.3).

	IGF-I Normal Death rates per 1000 (no. of deaths)	IGF-I High Death rates per 1000 (no. of deaths)
GH < 2 µg/L	9.16 (20)	11.21 (2)
GH > 2 µg/L	18.19 (16)	25.14 (22)

Table 3.3: Crude Death Rates per 1000 (number of deaths) in groups defined by GH status and serum IGF-I levels

3.5.4 Effect of hypopituitarism on mortality

Internal comparison of patients with or without evidence of hypopituitarism (one or more hypothalamic-pituitary axes deficient *versus* all hypothalamic-pituitary axes intact) did not identify any effect on outcome (rate ratio 1.27, [0.81-2.02]; p=0.3), although as demonstrated in Table 3.4, there was a trend towards increased mortality in those with the greater number of deficient hypothalamic-pituitary axes (p=0.07).

3.6 Discussion

Evidence from several studies has suggested that the increased mortality associated with untreated acromegaly is improved if GH secretion is reduced to less than 2.5 µg/L, whether measured as the mean of a GH day profile (30) or a random GH level (31,45).

Number of deficient axes	Rate ratio (95% CI)
1 axis v 0 axes	1.10 (0.65-1.87)
2 axes v 0 axes	1.40 (0.71-2.78)
3 axes v 0 axes	1.69 (0.88-3.22)

Table 3.4: The impact of the degree of hypopituitarism on mortality in patients with acromegaly (p = 0.07)

These studies have arbitrarily adopted a cut off point of 2.5 μg/L to define an adequate response to treatment, with little scientific basis to this selection. In this large study, comparison of crude death rates per 1000 population suggests that a GH of 2 μg/L measured either as the mean of a GH day profile or across an oral glucose tolerance test, or a random GH level, may be a more appropriate treatment target. Mortality was increased in the subgroup of patients with GH levels > 2 μg/L.

There is also evidence from this study that younger patients may be at greater risk from exposure to levels of GH > 2 μg/L. This corroborates the findings of others who have also demonstrated higher mortality rates in younger patients (45). The ability to select patients who may benefit most from intervention with effective, but expensive, medical treatments, is potentially of great value in clinical practice.

The presence or absence of hypopituitarism (one or more hypothalamic-pituitary axes deficient) did not have any effect on outcome, but there was a trend towards reduced survival in those with the greatest number of deficient axes.

In patients with pituitary tumours, excluding those secreting growth hormone, hypopituitarism is associated with increased mortality (134). This effect has been widely attributed to adult GH deficiency, although there is little direct evidence to support this conclusion. In our study of patients with GH excess, there is again some evidence to suggest that hypopituitarism is detrimental, arguing against a role for GH deficiency. Indeed, we have recently examined the impact of hypopituitarism on mortality in an updated version of the West Midlands Acromegaly database containing details of 501 patients. Patients with ACTH deficiency and gonadotrophin deficiency had a significantly increased SMR but patients with TSH deficiency did not (Table 3.5) (Sherlock *et al*., unpublished data). However on internal analysis, having adjusted for sex, attained age, calendar period, period of follow up and radiotherapy, only ACTH deficiency was associated with significantly increased mortality (RR 1.7, [1.2, 2.5], p=0.004), (Table 3.6). Further analysis revealed that increasing doses of hydrocortisone were associated with an increasing SMR (p for linear trend <0.001) (Table 3.7). On internal analysis, having adjusted for age, sex, calendar period, period of follow up and radiotherapy, there was a significant increase in RR of mortality in patients receiving hydrocortisone daily doses between 25 and 30 mg (RR 1.6, [1.1, 2.4], p=0.014) and hydrocortisone daily doses >30mg (RR 2.9, [1.4, 5.9], p=0.003), (Table 3.7). On internal analysis, there was a significant association between increasing dose of hydrocortisone and mortality as assessed by the likelihood ratio test for linear trend in relative risks (p=0.002). The main cause of death in the higher dose hydrocortisone group was cardiovascular disease. In the group of patients who were ACTH replete, 26.2% of deaths were due to cardiovascular causes.

Factor	Observed	Expected	SMR	95% CI	p value
ACTH					
Normal	69	53.8	1.3	1.0, 1.6	
Deficient	62	25.1	2.5	1.9, 3.2	<0.0005
TSH					
Normal	93	57.1	1.6	1.3, 2.0	
Deficient	42	19.7	2.1	1.5, 2.9	0.15
Gonadotrophins					
Normal	40	28.8	1.4	0.99, 1.9	
Deficient	66	31.4	2.1	1.6, 2.7	0.037

Table 3.5: Effect of pituitary axis deficiency on mortality compared to the general population, standardised for sex, attained age and calendar period. ACTH = adrenocorticotropic hormone, TSH = thyroid stimulating hormone. (O = Observed, E = Expected, SMR = Standardised Mortality Ratio, CI = Confidence Interval).

In the overall group of ACTH deficient patients, 31.6% of patients died from cardiovascular causes and there was an increase in the proportion of cardiovascular deaths with increasing hydrocortisone dose (Table 3.8).

Patients with Cushing's disease have been reported to have a cardiovascular SMR of 5 (136), which is due to a combination of abnormalities in blood pressure, glucose and lipid metabolism and the coagulation system (137).

Factor	RR	95% CI	p value
ACTH			
Normal	1		
Deficient	1.7	1.2, 2.5	0.004
TSH			
Normal	1		
Deficient	1.0	0.7, 1.4	0.829
Gonadotrophins			
Normal	1		
Deficient	1.2	0.8, 1.8	0.433

Table 3.6: Internal analysis of the effect of pituitary axis deficiency on mortality, adjusted for radiotherapy, follow-up time, sex, attained age, and calendar year. ACTH = adrenocorticotropic hormone, TSH = thyroid stimulating hormone. (RR = Relative Risk, CI = Confidence Interval).

Increased cardiovascular event rate (RR 2.56, CI 2.18-2.99) has also been described in patients receiving high doses of glucocorticoids (prednisolone >7.5 mg/day) (138). Traditionally the daily dose of hydrocortisone administered to patients with ACTH deficiency was 30 mg per day split into two doses. However, in recent years it has been reported that the cortisol production rate in normal subjects is less than was previously thought, equivalent to 5.7 mg/m2/day or approximately 9.9 mg/day (139). This suggests patients have been receiving supraphysiological glucocorticoid doses. Filipsson *et al*. (140) have described an adverse metabolic profile in a cohort of GH deficient patients on higher doses of glucocorticoid replacement. They found that

patients on hydrocortisone replacement had increased total cholesterol, triglycerides, waist circumference and HbA1c compared to the ACTH sufficient patients.

These findings raise the possibility that iatrogenic Cushing's syndrome may be contributing to the vascular mortality in patients with acromegaly who develop ACTH deficiency. Further studies are required to validate these findings.

Hydrocortisone daily dose	**SMR**	**95% CI**	**p value**
None	1.35	1.1, 1.7	0.006
<25mg	2.26	1.4, 3.7	0.0011
≥25mg	2.82	2.2, 3.7	<0.00001
	RR	**95% CI**	**p value**
None	1		
0 < HC ≤ 20	1.3	0.7, 2.6	0.439
20 < HC ≤ 25	1.4	0.6, 3.3	0.429
25 < HC ≤30	1.6	1.1, 2.4	0.014
HC > 30	2.9	1.4, 5.9	0.003

Table 3.7: Effect of increasing dose of hydrocortisone replacement of mortality in patients with acromegaly compared to the general. Linear trend in SMR of mortality with increasing dose of HC therapy, p value for linear trend <0.001. Internal analysis of the effect of increasing daily hydrocortisone replacement doses on mortality in patients with acromegaly. Likelihood Ratio Test for Linear Trend in relative risks p=0.002.

Hydrocortisone dose (mg)	Cardiovascular death (%)
$0 < HC \leq 20$	10
$20 < HC \leq 25$	33.3
$25 < HC \leq 30$	38.5
$HC > 30$	44.4

Table 3.8: Proportion of deaths due to cardiovascular causes with increasing hydrocortisone dose

IGF-I levels have been proposed as a first line investigation for the diagnosis and therapeutic monitoring of acromegaly (141,142). Indeed, the introduction of GH-antagonists as medical treatment for acromegaly necessitates the use of IGF-I in the biochemical monitoring of patients treated with these agents. However, few studies, with small numbers of deaths, have attempted to examine the role of IGF-I as a marker of long-term outcome (Table 3.9). In the first of these studies (162 patients, 12 deaths), those patients who were surgically cured, defined by a normal IGF-I in 82%, had mortality similar to that of the general population of the United States, while those with active disease as defined by a persistently elevated IGF-I had reduced life expectancy for the period that the IGF-I was elevated (32). In the study by Beauregard *et al*. (103 patients, 18 deaths), the impact of IGF-I on mortality is less clear, as the association between IGF-I alone and mortality was not reported (60). A further study also concluded that IGF-I normalisation reduced mortality to expected levels (51), however serum IGF-I was not an independent predictor of mortality when both GH and IGF-I measurements were included in the multivariate analysis, and was only significant when looking at SD scores >2 for IGF-I compared to normal IGF-I levels.

Further issues raised included the use of different IGF-I assays over the study period and a relatively small number of deaths. In our much larger study, and the subsequent Finnish Nationwide Survey of Mortality in Acromegaly (89), with a combined total of 151 deaths in 753 patients, there was no increase in mortality in the subgroup of patients with raised serum IGF-I levels (RR 1.2, [CI 0.71-2.02, p=0.05] and 0.46 [CI 0.17-1.26, p=0.13] respectively), suggesting last available serum IGF-I may not be as reliable a marker of mortality in acromegaly as last available GH.

Reference	Patients	Deaths	SMR	Predictive factors
Swearingen *et al.,* 1998	149	12	1.16	SMR 0.84 if IGF-I normal
Biermasz *et al.,* 2004	164	28	1.33	High IGF-I RR 4.78
Holdaway *et al.,* 2004	208	72	1.22	SMR 3.5 if IGF-I SD score >2
Mestron *et al.,* 2004	1219	56	NA	41 *versus* 15 deaths if IGF-I never normal (p=0.001)
K-Makelin *et al.,* 2005	334	56	1.16	IGF-I not predictive

Table 3.9: Studies assessing the role of IGF-I on mortality in acromegaly (RR=relative risk, NA = not available)

There are a number of potential explanations for this finding; IGF-I is bound to specific serum binding proteins whose levels are influenced not only by GH but also by non-GH dependent mechanisms such as sex steroids, insulin secretion and nutrition (143). The relationship between

serum GH and IGF-I is linear only up to GH values of approximately 12.5 μg/L; beyond this IGF-I levels plateau resulting in a curvilinear relationship (144). In addition, our study, like others, has shown that there is a considerable degree of discrepancy between GH and IGF-I when using clinically relevant cut off points such as GH values of less than 2-2.5 μg/L, which are known to be associated with improved outcomes (144,145). Bates *et al*. found that 9/24 (37.5 %) patients with GH levels of less than 2.5 μg/L still had elevated IGF-I levels, whilst a much smaller number of patients, 3/56 (5%) had normal IGF-I levels despite persistently elevated serum GH (> 2.5 μg/L). The cohort studied by Kaltsas *et al*. (145) displayed similar discrepancies, with persistently elevated IGF-I levels in 3 out of 23 patients (13%) with GH values less than 2.5 μg/L and normal IGF-I levels in 8 out of 44 patients (18%) with GH >2.5 μg/L. In our study, 10% of the cohort had GH values less than 2 μg/L but persistently elevated age-related IGF-I levels, whilst 19 % of the cohort had GH greater than 2 μg/L but normal age-related IGF-I levels. 34 of the 69 patients with normal age-related IGF-I and elevated GH were female, 25 of them postmenopausal; only 8 were on oestrogen replacement. This is relevant, as oestrogens modulate the secretion and the action of GH (146). Observational studies suggest that the presence of oestrogen is associated with GH resistance. GH, but not IGF-I levels, are higher in young women than in age-matched men (147). IGF-I levels are lower in GH-deficient women and the IGF-I increase in response to GH treatment is about half that of their male counterparts, resulting in women requiring a higher replacement doses of GH than men to achieve similar IGF-I levels (148). The route of administration is a major determinant of the effect of oestrogen on the GH/IGF-I axis (149). When administered orally, the liver is exposed to pharmacological oestrogen concentrations, inhibiting IGF-I production, an effect that is avoided by the parenteral route (150). In acromegaly, there is a gender difference in the relationship between GH output

and IGF-I, with IGF-I being lower in women for a given GH concentration (151). These observations suggest gonadal status and mode of oestrogen replacement may play a role in the GH/IGF-I discrepancy seen in female patients treated for acromegaly and clinicians need to be mindful of the influence of sex steroids when evaluating GH status.

Thus, although at defined cut off values there is a correlation between GH and IGF-I concentration, there are marked discrepancies between cured or safe GH values and normal IGF-I concentrations. This may reflect the fact that not all actions of GH are mediated by IGF-I (152) or conversely that IGF-I is not the only growth factor regulated by GH (153). In addition, many factors other than GH contribute to the determination of serum IGF-I, including nutritional state, liver function, serum protease activity, IGF-I-binding proteins and sex hormones (143). The concept of separate endocrine *versus* paracrine compartments of IGF-I is supported by the observation that GH receptor mutant mice have no measurable IGF-I in the circulation, but have normal expression of IGF-I mRNA in peripheral tissues (154). Studies in tissue-specific knockout mice have demonstrated that even when circulating levels of IGF-I are reduced by around 75%, postnatal growth and development can be normal, suggesting local tissue-specific IGF-I may be contributing to growth and development independently of GH action (155).

Other proposed mechanisms to explain the discrepancy between GH and IGF-I include persistent GH dysregulation following “cure” (156), alteration of the normal IGF-I/GH relationship by somatostatin analogues (157) and disruption of somatostatin tone due to radiotherapy (158). Our data indicate that the benefits of reducing serum GH to < 2 μg/L outweigh the benefits of reducing serum IGF-I to normal and this should therefore remain the principal target of treatment. However, there is a suggestion that normalisation of IGF-I may confer a small additional benefit.

The benefits of reducing serum GH discussed in this study pertain predominantly to mortality, but previous studies have demonstrated persistent morbidity including cardiac disease (42) and sleep apnoea (159), even when serum GH levels of less than 2.5 μg/L are achieved. This has led to speculation as to whether this is due to irreversibility of symptoms and signs or continuous low-grade GH hypersecretion (160). However, before current criteria for remission are altered, further research is required to determine the levels to which serum GH can be suppressed without inducing hypopituitarism in patients with acromegaly.

The estimated duration of the disorder prior to treatment has been shown to be a significant independent predictor of mortality in acromegaly, no doubt reflecting cumulative exposure to excessive levels of GH and IGF-I (51). However, most studies addressing mortality in acromegaly have used last GH and IGF-I levels for analysis. Assuming that the mechanism of damage to organs and tissue is a chronic process, it may be more appropriate to use cumulative GH and IGF-I exposure rather than latest values to assess impact on mortality. The longer an individual is exposed to excessive levels of GH, the more the damage and the greater the risk of death. It would therefore be logical to quantify GH exposure in terms of Growth Hormone Unit (GHU) – Years. For example, if an individual is exposed to 10 μg/L for 2 years then this corresponds to 20 GHU-years. If the individual is exposed to 5 μg/L for the next 6 months, then this corresponds to 2.5 GHU-years. If the individual is exposed to 3 μg/L for the next 6 months, then this corresponds to 1.5 GHU-years. So in total for this three year period the individual has accumulated 24 GHU-years. In collaboration with Professor Mike Hawkins and Dr Raoul Reulen of the Centre for Childhood Cancer Survivor Studies, Department of Public Health & Epidemiology, University of Birmingham, we recently compared the relative risk of mortality in 501 patients with acromegaly based on cumulative GH exposure. We adjusted for pre-treatment

GH exposure by assuming that at diagnosis, the patient has been exposed to the pre-treatment GH level for 8 years. This was based on a mean delay in diagnosis of around 8.7 years reported by Molitch (161). These data were used to calculate an "instantaneous" GH level, taking into account total GH exposure (Sherlock *et al*., unpublished data). The impact of instantaneous GH level on all cause mortality adjusted for attained age, sex, calendar period, period of follow-up, pre-treatment GH and radiotherapy is shown in Table 3.10. The Likelihood Ratio Test for linear trend in relative risks was significant with p=0.019. Table 3.11 shows the differences in relative risk taking different dichotomous cut-points in the instantaneous exposure. There was a trend towards increased mortality when instantaneous GH was >1.0 μg/L compared to ≤1.0 μg/L, although this did not achieve statistical significance due to the small number of deaths.

Instantaneous GH (μg/L)	**Relative risk**	**95%CI**	**P-value**
GH<1	1		
1≤GH<2.5	1.6	(0.9, 2.9)	0.131
2.5≤GH<5	1.3	(0.7, 2.6)	0.372
5≤GH<10	1.9	(1.0, 3.6)	0.042
10≤GH<50	2.1	(0.4, 10.1)	0.363
50≤GH	7.1	(1.9, 27.0)	0.004

Table 3.10: Effect of instantaneous GH level on all cause mortality adjusted for attained age, sex, calendar period, period of follow-up, pre-treatment GH and radiotherapy

GH	No of Deaths	No of Patient years	Relative risk	95%CI	P-value
≤0.5µg/L	14	831	1		
>0.5µg/L	93	4590	1.3	(0.7, 2.4)	0.323
≤1.0µg/L	22	1530	1		
>1.0µg/L	85	3891	1.6	(0.9, 2.6)	0.085
≤1.5µg/L	30	1968	1		
>1.5µg/L	77	3452	1.4	(0.9, 2.2)	0.150
≤2.0µg/L	37	2368	1		
>2.0µg/L	70	3053	1.3	(0.9, 2.1)	0.181
≤2.5µg/L	46	2717	1		
>2.5µg/L	61	2704	1.2	(0.7, 1.8)	0.520
≤5.0µg/L	66	3623	1		
>5.0µg/L	41	1798	1.3	(0.8, 2.1)	0.247

Table 3.11: Effect of instantaneous GH level on all cause mortality – dichotomous exposure with varying cut-points

This method of analysis has the advantage that it takes into account the cumulative impact of GH exposure, which has been shown to be clinically relevant. However, there are a number of flaws, not least the assumed duration of GH exposure prior to diagnosis and treatment. Although these data are interesting, they need to be validated by future studies.

A number of problems arise when using biochemical targets to determine clinical outcome. Both IGF-I and GH assays, even those in use today, are prone to large variability. Pokrajac *et al*. demonstrated more than two-fold variation in GH and IGF-I values measured in different laboratories (162). Over time, the measurement of GH has evolved from polyclonal radioimmunoassays of limited sensitivity to two-site monoclonal antibody, non-isotopic assays

with enhanced sensitivity, allowing accurate quantification of previously undetectable GH values. The 2000 consensus statement defined the criteria for remission of acromegaly as a normal age-matched serum IGF-I level and a GH nadir of less than 1 μg/L during an oral glucose tolerance test (142). While some series have supported the GH cut off of 1 μg/L, other data have shown that it may be too high and will miss some patients with persistent active disease following treatment (163). Recent studies using new highly sensitive assays indicate that for control to be achieved, the nadir GH level during OGTT should be considerably below 1 μg/L (164), and a recent consensus statement defined remission as a GH value below 0.4 μg/L (165). GH levels measured by highly sensitive assays are significantly lower than those measured by polyclonal radioimmunoassay (164); we therefore cannot apply criteria establishing disease remission in acromegaly that were derived with older assays to GH levels measured with many assays in current use. The recent consensus statement on the standardisation of GH assays (86) will almost certainly lead to a major revision of GH targets for monitoring treatment success and reducing mortality rates in acromegaly. Until further data are available, clinicians need to be aware of the more sensitive assays in use in their laboratories and the normal GH nadir values achieved during OGTT using these assays.

In conclusion, these results suggest that a GH value of 2 μg/L should be regarded as an appropriate therapeutic target, as values above this level are associated with increased mortality. Using the newer assay now in use in our laboratory, this would equate to a reading of around 1.3 μg/L. A raised IGF-I level does not adequately predict increased mortality, suggesting that the recommendation to use IGF-I as the sole biochemical marker of "cure" is premature. ACTH deficiency and glucocorticoid replacement may play a role in mortality in acromegaly, but further

studies are required to define the contribution of hypopituitarism to any increase in mortality in patients with acromegaly.

4. UNDERLYING EXPLANATION FOR INCREASED MORTALITY IN ACROMEGALY- OTHER FACTORS INCLUDING RADIOTHERAPY

4.1 Introduction

In Chapter 3 I described the impact of hormonal factors on mortality in acromegaly. Other factors may also influence mortality in acromegaly and these will be examined in detail in this section. A number of studies in patients with pituitary tumours have suggested that radiotherapy may be associated with an increase in cerebrovascular morbidity and mortality (166,167), although patients with acromegaly have been universally excluded.

I examined the outcome of patients with acromegaly who received radiotherapy amid these ongoing concerns.

Analysis of the determinants for mortality in acromegaly indicates that approximately 60% of patients die from cardiovascular disease, 25% from respiratory disease, and in 15% of patients, the cause of death is attributed to malignancy (46). From published retrospective studies, the major negative determinants for survival are high GH levels and the presence of hypertension, cardiac disease and diabetes mellitus (46). Longer symptom duration, which is a surrogate marker for duration of exposure to excess GH, is also linked to poor outcome (51).

Hypertension and glucose intolerance are important contributory factors to the vascular morbidity associated with acromegaly (168). However, there are few published reports on their impact on mortality in acromegaly and how this correlates with GH and IGF-I levels. Using data from the West Midlands Acromegaly database, I examined the impact of hypertension, diabetes mellitus

and ischaemic heart disease on mortality in acromegaly and explored the association between these outcomes and GH / IGF-I levels.

4.2 Patients

The West Midlands Acromegaly Database was established in 1990 and on 31st December 2001 contained demographic and clinical details of 419 patients (241 female) with acromegaly from 16 referral centres across the West Midlands Region. The database and patient cohort are described in detail in Section 2.2.

4.3 Endocrine evaluation

Assay and measurement details are outlined in Section 2.3. Serum GH levels were measured by an in-house RIA at the Regional Endocrine Laboratory at the University Hospital Birmingham, Selly Oak, as previously described (85). Serum IGF-I was measured using an in-house RIA with acid-ethanol extraction performed to remove IGF binding proteins, as previously described (67).

4.4 Statistical analysis

Statistical analysis was performed by Dr Michael Hills, Senior Lecturer (retired), Department of Medical Statistics, London School of Hygiene and Tropical Medicine. An external comparison of the entire cohort with the general population was made using the Standardised Mortality Ratio (SMR) based on published mortality data for England and Wales by 5-year age and calendar

periods as described in Chapter 2. Internal comparisons between groups were made, using the ratio of mortality rates (RR), obtained with the statistical package Stata (Stata Corp. 2001, Stata Statistical Software: Release 7.0. College Station, TX: Stata Corporation) using Poisson regression to control for age and sex. Multiple exponential regression was used to determine the effect of radiation therapy.

Measurements of GH and IGF-I are expressed as the mean ± SEM and the Logrank test was used to examine the impact of hypertension, diabetes mellitus and ischaemic heart disease on mortality. The Mann-Whitney test was used to compare differences in mean GH and IGF-I between patients with and without hypertension, ischaemic heart disease and diabetes mellitus. A value of $p < 0.05$ was considered statistically significant.

4.5 Results

4.5.1 Effect of radiotherapy on mortality

The use of external radiotherapy (total dose ranging from 45-50 Gy in 30 treatments via 3 ports) was associated with increased mortality. Table 4.1 shows the cause-specific SMRs for subjects treated with radiotherapy. There is a large excess in all-cause mortality in patients who had received external radiotherapy, compared to the general population (SMR = 59/37.4 = 1.58, [1.22-2.04]; p=0.005). This was predominantly due to cerebrovascular disease (SMR = 16/3.6 = 4.42, [2.71-7.22]; p=0.005), although there was also a small increase in cardiovascular mortality (SMR = 20/12.5 = 1.60, [1.03-2.48]; p=0.096).

Cause	Observed Deaths	Expected Deaths	SMR (95% CI)	P
All cause	59	37.4	1.58 (1.22-2.04)	0.005
Cerebrovascular	16	3.6	4.42 (2.71-7.22)	0.005
Cardiovascular	20	12.5	1.60 (1.03-2.48)	0.096
Respiratory	7	4.0	1.75 (0.84-3.68)	0.261
Malignancy	12	12.0	1.00 (0.57-1.76)	1.000

Table 4.1: All cause and cause specific mortality in patients with acromegaly treated with radiotherapy

Internal comparison of 211 patients who received external radiotherapy with 206 who did not, controlled for age and sex, confirmed a poor outcome in the former with a rate ratio of 1.67, (1.1-2.56; p=0.02). This effect was consistent despite controlling for the effects of GH, IGF-I, tumour size (macroadenoma/microadenoma), tumour extension (beyond sella) or hypopituitarism (any deficient axis).

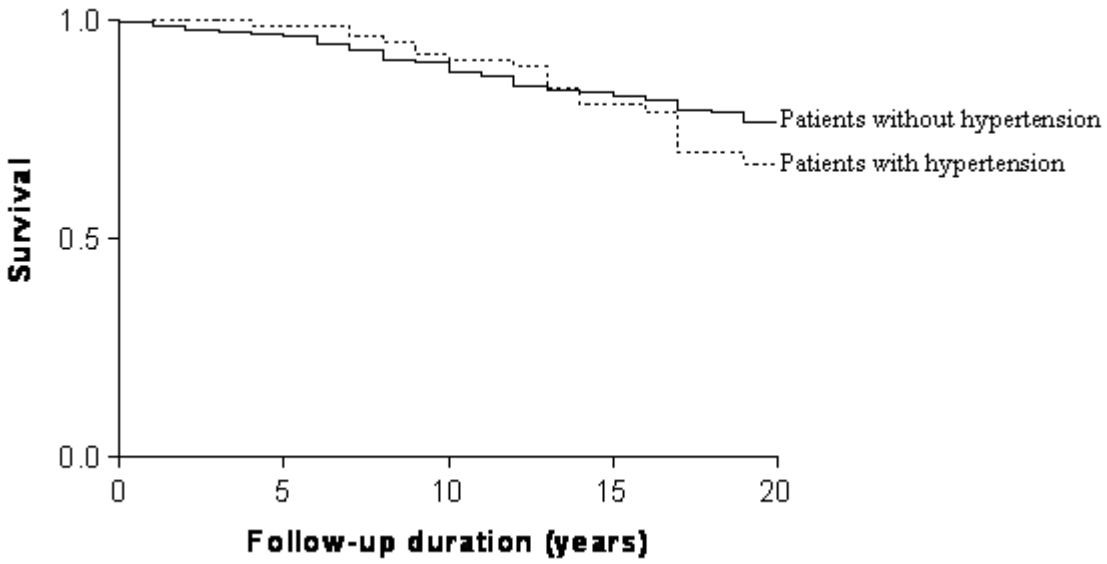
4.5.2 Effect of comorbidities on mortality

Hypertension was diagnosed prior to or during the study period in 100 patients (58 female); of these, 74 were alive and 26 deceased. Diabetes mellitus was diagnosed in 36 patients (23 female), 23 alive and 13 deceased, and ischaemic heart disease was diagnosed in 24 patients (13 female), 16 alive and 8 deceased. There was no difference in survival between patients with and without hypertension (Figure 4.1) or between those with and without ischaemic heart disease (Figure 4.2).

There was a trend towards increased mortality in patients with ischaemic heart disease, but this did not attain statistical significance. However the presence of diabetes mellitus significantly reduced survival (Chi square 4.52, $p = 0.03$), with a hazard ratio of 1.85 (CI 1.07-4.86) (Figure 4.3).

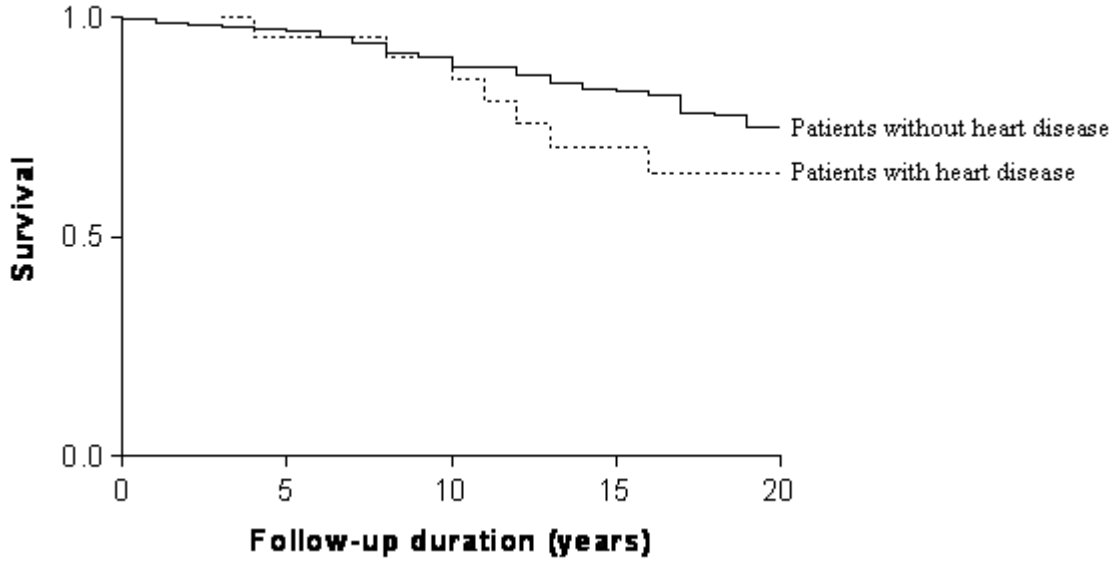
Evaluation of mean post-treatment GH and IGF-I concentrations revealed no statistically significant difference between patients with and without hypertension (Table 4.2). By contrast, mean post-treatment GH concentration was higher in the cohort of patients with ischaemic heart disease than in those without (10.4 ± 6.5 µg/L *versus* 4.7 ± 0.6 µg/L, $p < 0.05$), but mean IGF-I concentrations were not significantly different (311 ± 37 µg/L *versus* 283 ± 12 µg/L, $p = 0.40$). Mean post-treatment GH concentration was also higher in patients with diabetes mellitus than in those without (6.0 ± 1.2 µg/L *versus* 5.0 ± 0.7 µg/L, $p = 0.007$); however, there was no significant difference in IGF-I levels between the two groups (357 ± 47 µg/L *versus* 278 ± 12 µg/L, $p = 0.20$).

Figure 4.1



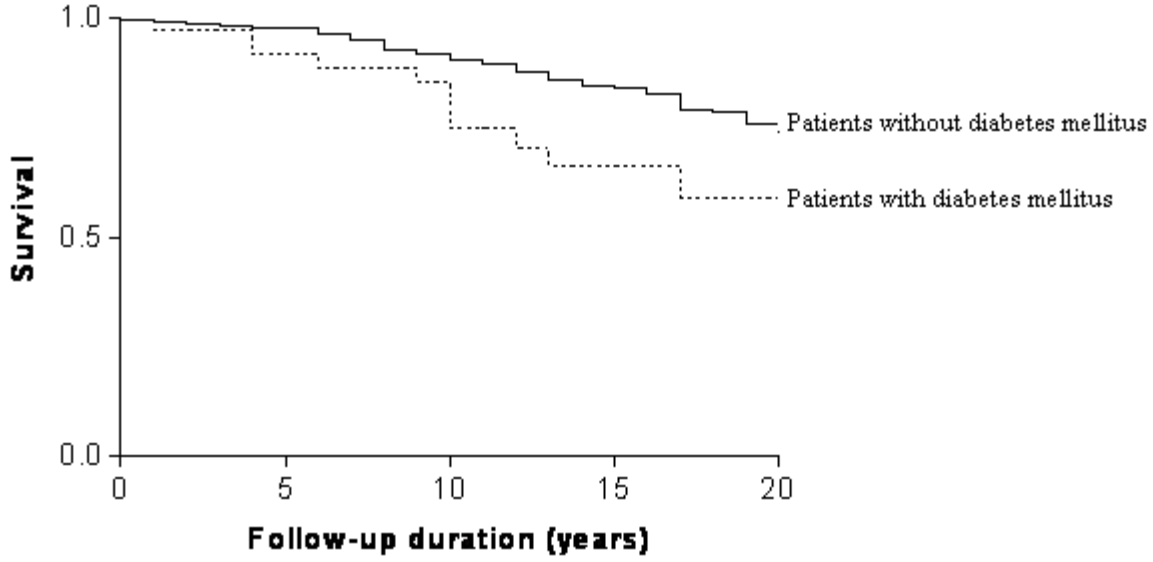
Mortality in acromegaly in patients with and without hypertension (p = 0.30)

Figure 4.2



Mortality in acromegaly in patients with and without ischaemic heart disease (p = 0.08)

Figure 4.3



Mortality in acromegaly in patient with and without diabetes mellitus (p = 0.03)

Patient comorbidities	Mean GH ± SEM (µg/L)	Mean IGF-I ± SEM (µg/L)
Hypertension		
Yes	3.8 ± 0.7	277 ± 23
No	5.5 ± 0.9	288 ± 13
Ischaemic heart disease		
Yes	10.4 ± 6.5	311 ± 37
No	4.7 ± 0.6*	283 ± 12
Diabetes mellitus		
Yes	6.0 ± 1.2	357 ± 47
No	5.0 ± 0.7**	278 ± 12

Table 4.2: Mean post-treatment GH and IGF-I concentrations in patients with and without hypertension, ischaemic heart disease and diabetes mellitus ($p < 0.05$, ** $p = 0.007$)*

4.5.3 Other factors affecting survival

Neither tumour size (macroadenoma *versus* microadenoma) nor tumour extension (beyond the sella *versus* within the sella) had any impact on survival. For the former, the rate ratio, controlled for age and sex, was 1.11 (0.61-2.01; p=0.74) and for the latter 1.42 (0.85-2.38; p=0.18).

4.6 Discussion

A number of studies in patients with pituitary tumours have suggested that radiotherapy may be associated with an increase in mortality (166,167), although patients with acromegaly have been universally excluded. This is the first study to show that patients with acromegaly are also subject to reduced life expectancy following pituitary radiotherapy. This effect was consistent despite controlling for the effects of serum GH, serum IGF-I, tumour size (macroadenoma/microadenoma), tumour extension (beyond sella) or hypopituitarism (any deficient axis). The strength of the association between increased mortality and radiotherapy is further enhanced by the fact that the predominant cause of death in this group of patients was cerebrovascular disease. In the Finnish Nationwide Survey of Mortality in Acromegaly, treatment with radiotherapy was also associated with increased mortality (89). Of 334 patients with acromegaly, 116 had been treated with radiotherapy. The standardised mortality ratio for irradiated patients was 1.69 (95% CI 1.05-2.58, $p<0.001$), which was significantly higher than in the general population, whilst the standardised mortality ratio for those not treated with radiotherapy was 0.94 (95% CI 0.62-1.37). Cerebrovascular disease was a common cause of death among patients who had been treated with radiotherapy. Data from the Spanish Acromegaly Study also examined the link between radiotherapy and mortality (52). Patients who died were twice as likely to have been treated with radiotherapy as those who survived (Table 4.3).

Several authors have reported cerebrovascular complications in patients receiving radiotherapy for central nervous system tumours. These include cases of documented arteriographic changes within the radiation fields in children and adults suffering strokes following irradiation (169,170)

and Moya-moya syndrome with severe stenosis or occlusion of the internal carotid arteries (171). In a series of 156 patients receiving radiotherapy for non-functioning pituitary adenomas, the incidence of cerebral infarction was found to be elevated in those treated with higher biological equivalent doses (166). Similarly, in another study of 331 patients with pituitary adenomas treated with surgery and radiotherapy, increasing doses of radiotherapy were associated with increasing risk of cerebral infarction (172). In this study, the relative risk of first cerebrovascular accident compared to the general population was 4.1 (CI 3.6-4.7). Debate surrounds the exact cause of the increased cerebrovascular risk seen in patients treated with radiotherapy, but it is thought that radiation may cause a variety of vascular injuries and haemodynamic changes to the cerebral vasculature (173). Radiation leads to damage of both large and small vessels, but has a predilection to smaller vessels (174). The vasculature is vulnerable as endothelial cells are radiosensitive, which leads to several ultrastructural changes, with resultant increased capillary permeability and intracellular oedema which may be followed by platelet and fibrin thrombosis. Larger lesions in arterioles can also occur, leading to myointimal proliferation, foamy macrophage plaques, fibrinoid necrosis of the media or hyalinisation of the media, leading to narrowing of the vessel lumen (174).

Not surprisingly, the increased incidence of cerebrovascular disease seen in patients treated with pituitary radiotherapy is reflected in an increase in cerebrovascular mortality in these patients. Having determined the incidence of cerebrovascular accidents in a cohort of patients with predominantly non-functioning pituitary adenomas treated with surgery and radiotherapy (172), Brada *et al*. went on to assess cerebrovascular mortality within the group (167). In the cohort of 334 patients representing a total of 4982 person-years, 79 percent had been treated with transcranial or transsphenoidal surgery and all patients had received radiotherapy. Deaths from

cerebrovascular disease accounted for 26% of the total. There were 33 deaths from cerebrovascular disease, compared with 8.04 expected, leading to an estimated relative risk of death from cerebrovascular disease of 4.11 (CI 2.84-5.75). There was a statistically significant difference in the relative risk of cerebrovascular deaths in women (RR 6.93 [95% CI 4.29-10.60]) compared with men (RR 2.4 [95% CI 1.24-4.20]). The relative risk of cerebrovascular deaths in patients with non-functioning tumours was 3.65 [95% CI 2.26-5.58], compared with 5.23 [95% CI 2.25-10.30] in patients with hormonally active tumours.

Other factors may also contribute to mortality following pituitary radiotherapy. Hypopituitarism occurs almost invariably following pituitary radiotherapy; over 50 percent of patients treated with pituitary radiotherapy will develop deficiencies in one or more anterior pituitary hormones over the following decade (175-177). The speed of onset of hypopituitarism is related to the dose of radiotherapy, and the incidence increases with time from treatment (175,176). A number of studies have examined mortality in patients with hypopituitarism and found increased mortality compared with age-matched controls, predominantly due to cerebrovascular and cardiovascular disease (134,178-180). The overall standardised mortality ratio in these studies was around 2, with females appearing to be more severely affected. Of a total of 1863 patients included in these studies, nearly 50% had been treated with pituitary radiotherapy. In two of these studies, treatment with radiotherapy was not associated with increased mortality (178,179), and in the third, as almost all patients had received radiotherapy post-operatively, any possible contribution of radiotherapy to the increased cerebrovascular mortality could not be evaluated (180). However, in the large prospective study from the West Midlands region of the United Kingdom, comprising over 1000 patients and 181 deaths, treatment with radiotherapy was associated with a significantly increased mortality rate (134). Standardised mortality ratio was 2.32 (99% CI 1.71-

3.14, p=0.004) in the 353 patients with hypopituitarism that had been treated with cranial radiotherapy, compared to 1.87 (99% CI 1.62-2.16) in the general cohort of patients with hypopituitarism. The excess mortality was caused to a significant degree by a marked increase in cerebrovascular deaths in patients who underwent radiotherapy (SMR 4.36 [99% CI 2.48-7.68, p=0.001]).

While the studies above confirm the excess mortality seen in patients with hypopituitarism, no clear answer has emerged with regards to causal relation. It is difficult to be certain about the individual contribution of various risk factors in the very heterogeneous population of hypopituitary patients. As well as the direct effects of radiation on cerebral vasculature described above, some authors have suggested that deficiencies in specific pituitary hormones may contribute to the increased vascular mortality seen in these patients. Growth hormone secretion is the most vulnerable of the anterior pituitary hormones following radiation damage to the hypothalamo-pituitary axis, followed by the gonadotrophins (175,181). Based on the assumption that most patients with hypopituitarism would be growth hormone deficient, Rosen and Bengtsson speculated that growth hormone deficiency might explain the premature death from vascular disease seen in their series; however, no evidence was available to support this speculation (178). By contrast, the only endocrine factor implicated in the excess mortality in the West Midlands study was untreated gonadotrophin deficiency, with sex steroid replacement significantly reducing mortality (134).

A recent study examining risk factors for cerebrovascular mortality in 342 patients with pituitary disease treated with surgery and radiotherapy provides a useful contribution to the field (182). The study compared radiation regimens and duration of symptoms of hypopituitarism between 31 subjects who died from cerebrovascular disease and a matched control group of 62 patients from

the same cohort of hypopituitary patients who had not died from cerebrovascular disease. No significant differences were found between the two groups in maximum absorbed dose, maximum biological equivalent dose, field size or number of fractions. The only difference between the patients who died from cerebrovascular disease and the control group was the duration of symptoms of hypopituitarism, leading the authors to speculate that untreated hormone deficiencies may be more directly implicated in the increased cerebrovascular mortality seen in hypopituitarism than radiotherapy *per se*. Of interest, however, is that in all the patients who died from cerebrovascular disease, the lesion was localised within the irradiated area of the brain.

Other complications of pituitary radiotherapy have also been described, including the formation of secondary intracranial tumours (183,184), damage to the optic nerves (16,185) and impaired neurocognitive function (186). Many of these are the result of treatments from the past with antiquated techniques that applied much larger radiation doses to the treatment fields due to limited technology in localisation by imaging and to less conformal radiation delivery techniques (187). With current radiation therapy regimens, treatment-induced vision injury or blindness is uncommon. The generally accepted threshold of single fraction radiation tolerance to the optic system is 8–10 Gy (187). Older radiation delivery techniques have resulted in data suggesting a second tumour risk of 2–3% at 10–20 years following radiation treatment (187). However, once again with modern treatment regimens this has become a rare occurrence and does not significantly contribute to mortality in modern patient cohorts.

There is mounting evidence that in a subgroup of patients in whom surgery is unlikely to result in remission, long-term treatment with depot somatostatin analogues as primary therapy is a safe and effective option (188). In addition, emerging data suggest that somatostatin analogues may cause significant tumour shrinkage (71,72). This needs to be set against the undoubted benefit of

pituitary radiotherapy in patients with invasive pituitary tumours, but these data will result in a reappraisal of the current indications for external radiotherapy in patients with acromegaly.

Reference	Patients	DXT	SMR	RR	Comments
Biermasz et al, 2004	164	57 CRT	NA	1.73 (0.77-3.86) Age and sex adjusted 1.169 (0.52-2.65)	Cause of death not known
Holdaway et al, 2004	208	143 CRT 35 Yttrium	NA	NA	No increase stroke mortality
Kauppinen-Makelin et al, 2005	334	116 CRT	DXT group 1.69 (1.05-2.58) vs. 0.94 (0.62-1.37) for non DXT (p<0.001)	2.27 (p=0.08)???	6/8 stroke deaths
Mestron et al 2005	1219	504 CRT 27 stereotactic radiotherapy 9 radiosurgery	HR 2.29 (1.03-5.08)	NA	Cerebrovascular mortality data NA
Sherlock et al 2009	501	220 CRT 17 Yttrium/ radiosurgery	2.1 vs 1.4 for non DXT (p=0.006)	1.8 (p=0.008)	Cerebrovascular SMR 4.1

Table 4.3: Studies assessing the role of pituitary radiotherapy in mortality in patients with acromegaly (References (51,52,88,89), Sherlock et al. unpublished data)

In acromegaly, prolonged exposure to elevated GH and IGF-I levels results in significant morbidity affecting multiple organ systems. These complications not only have a significant impact on patients' quality of life, but also contribute to the 2- to 3-fold increased mortality seen in patients with acromegaly (29-31,34,45,51,60,84). In particular, the presence of hypertension, cardiac disease and diabetes mellitus have been shown to be major negative determinants for survival (46).

There are few studies assessing morbidity in acromegaly according to post-treatment GH or IGF-I and its ultimate impact on mortality. Assessment of this issue is complex, as many of the

chronic complications of the disorder such as arthritis and hypertension may persist despite biochemical remission.

In this study, using morbidity and mortality data from the West Midlands Acromegaly Database, we examined the impact of hypertension, diabetes mellitus and ischaemic heart disease on mortality in acromegaly and explored the association between these outcomes and GH/IGF-I levels. The presence of diabetes mellitus significantly reduced survival in this cohort of patients. There was a trend towards increased mortality in patients with ischaemic heart disease, however this did not attain statistical significance, possibly due to the small numbers. There was no difference in survival between patients with and without hypertension.

Hypertension occurs in around a third of all patients with acromegaly, ranging in some series up to 60% (38,189,190). The pathogenesis of hypertension in acromegaly is thought to be multifactorial, with an increase in the sodium pool, a decrease in atrial natriuretic peptide, the presence of insulin resistance and the direct effects of GH/IGF-I on vascular endothelial cells all playing a role (189). Hypertension is considered one of the most relevant negative prognostic factors for mortality in acromegaly (29,31,46,51). Wright *et al*. found that mean systolic and diastolic blood pressures were higher in a deceased group of patients with acromegaly than in those still living, and Rajasoorya *et al*. found hypertension to be a significant independent risk factor for mortality on multivariate analysis. In our study, the presence of hypertension did not result in reduced survival. The reasons for this discrepancy are unclear, although the heterogeneity of the study populations and the different methods used for blood pressure assessment may influence the findings.

GH and IGF-I receptors are expressed in cardiomyocytes (191) and IGF-I has been shown *in vitro* to cause hypertrophy of cultured cardiomyocytes and delay cardiomyocyte apoptosis

(192,193). The most common feature of acromegalic cardiomyopathy is concentric biventricular hypertrophy (35). Cardiac walls are generally thickened, but cardiac chambers are rarely enlarged due to relative increase of cardiac myocyte width (194). It is thought that cardiac hypertrophy occurs early in the course of the disease and worsens with increasing disease duration (195). Age and long duration of GH/IGF-I excess appear to be the main determinants of cardiac abnormalities; data from *in vivo* and post-mortem studies show a prevalence of cardiac hypertrophy > 90% in patients with long disease duration (35,196). However, some recent studies have demonstrated that structural changes of the heart can occur in patients exposed to GH hypersecretion for short periods (197,198). Cardiac hypertrophy initially results in increased systolic output, followed by diastolic dysfunction and exertional systolic dysfunction. Left untreated, this may progress to systolic dysfunction at rest and eventually heart failure (199). Cardiac dysfunction is more severe in patients with hypertension and diabetes mellitus (168). Results of the Framingham heart study indicate that an increase in left ventricular mass predicts a higher incidence of clinical events, including death attributable to cardiovascular disease (200). Whether this relationship is also present in patients with acromegaly is unknown, as there is no clear consensus regarding the prevalence of coronary artery disease in acromegaly, with estimates ranging from 3 to 37% in different series (201). However, cardiovascular disease is a leading cause of mortality in these patients and the presence of cardiac disease is associated with reduced survival (31,46). Most published data on the incidence of coronary heart disease (CHD) and atherosclerosis in acromegaly date back to post-mortem examinations in old series of patients (196,202). Among 27 patients with acromegaly who underwent autopsy, 11% had significant coronary artery disease, 15% had evidence of old myocardial infarction and 24% had significant atherosclerosis of the abdominal aorta (196). In a recent observational study, 39 patients with

acromegaly were evaluated for the risk of CHD through a combination of the Framingham score and detection of coronary artery calcium (CAC) content by computed tomography (203). Overall, the authors reported that 41% of patients with acromegaly were at risk for coronary atherosclerosis. This high estimated risk was not corroborated in a further study in which fifty-two consecutive patients with controlled or uncontrolled acromegaly were followed prospectively for 5 years (202). The likelihood for developing CHD was estimated using the Framingham scoring system (FS). Thirty-seven patients (71%) had low, 14 patients (27%) had intermediate and one patient (2%) had high CHD risk. CHD risk was unrelated to disease activity or estimated duration of disease. This was in contrast to our study, in which patients with ischaemic heart disease had higher GH concentrations than those without. Several other risk factors for myocardial ischemia such as elevated levels of lipoprotein-a, triglycerides, fibrinogen, plasminogen activator inhibitor and tissue plasminogen activator have been documented in patients with acromegaly (199), and the roles played by hypertension and altered glucose metabolism are discussed in greater detail elsewhere in this section. It is therefore difficult to isolate the contribution made by elevated GH levels alone to CHD risk. Data on the effects of GH/IGF-I on vascular structure and function in acromegaly are inconsistent; some studies have demonstrated early features of atherosclerosis, reporting an increase in carotid artery intima-media thickness (IMT) (204) and endothelial dysfunction (98,205). However, other studies have demonstrated lower carotid IMT compared to controls (206). In our study, we found a trend towards increased mortality in patients with ischaemic heart disease, but this did not attain statistical significance, possibly due to the small numbers (Figure 4.2). Mean post-treatment GH concentration was higher in the cohort of patients with ischaemic heart disease than in those without, but there was no difference in mean IGF-I concentrations between the groups.

Cardiac arrhythmias, including paroxysmal atrial fibrillation, paroxysmal supraventricular tachycardia and sick sinus syndrome are more common in patients with acromegaly than in controls (35,39). A high incidence of valvular disease has also been found in post-mortem (196) and echocardiographic (42) studies. However, neither of these has a significant impact on mortality in acromegaly.

GH excess induces insulin resistance by impairing the ability of insulin to suppress gluconeogenesis, decreasing peripheral glucose utilisation, and reducing insulin receptor numbers and binding affinity (35). The prevalence of overt diabetes mellitus ranges from 19 to 56% in patients with active acromegaly, with impaired glucose tolerance (IGT) occurring in up to 46% (35,43).

The presence of diabetes mellitus has been demonstrated to be a significant predictor of mortality in a number of studies (29,31,46), although other groups have found this not to be the case (30,51,91). In the study by Wright *et al*., clinical diabetes was present in 20% of the patient cohort, and was associated with increased mortality (29). The proportion of patients that died was greater in the group with clinical diabetes (54%) than in the group without (22%). Although both mortality and the prevalence of diabetes increase with age in the general population, the authors felt the reduced survival was directly associated with diabetes, as the ages of onset of acromegaly and diabetes were similar in both deceased and survivor groups. Rajasoorya *et al*. also demonstrated increased mortality in the presence of diabetes mellitus (31). Univariate analysis revealed that reduced survival was significantly associated with the presence of diabetes at diagnosis of acromegaly. In our cohort of patients, those with diabetes mellitus had significantly reduced survival compared to those without. Mean post-treatment GH concentrations were higher in the patients with diabetes mellitus (6.0 ± 1.2 µg/L *versus* 5.0 ± 0.7 µg/L, $p = 0.007$) which is

not surprising, as higher GH levels have been shown to be a risk factor for developing symptomatic diabetes mellitus (34).

Our findings suggest that a diagnosis of diabetes mellitus is a poor prognostic marker associated with increased mortality in patients with acromegaly. However GH levels, an independent predictor of outcome, were higher in patients with diabetes mellitus. In addition, impaired glucose metabolism has been shown to influence the severity of acromegalic cardiomyopathy; in 130 consecutive patients with acromegaly, those with impaired glucose tolerance and diabetes mellitus had significantly higher systolic and diastolic blood pressures than those with normal glucose tolerance (168). The prevalence of left ventricular hypertrophy in the subgroup of patients with hypertension and diabetes mellitus was 84.6%. This may contribute to the increased cardiovascular mortality seen in patients with acromegaly who have impaired glucose metabolism.

Although not assessed in this study, sleep apnoea syndrome (SAS) is a common finding in acromegaly, occurring in 45 to 80% of patients (207-210). In the general population, SAS has been shown to predispose to ischaemic heart disease, impaired glucose metabolism, hypertension and cerebrovascular accidents, all of which are common findings in patients with acromegaly (35,211,212). SAS may therefore represent an additional risk factor for cardiovascular disease and mortality in acromegaly. Obstructive SAS is the prevailing form in acromegaly, while the central and mixed types are less frequent (210). Craniofacial deformities, hypertrophy of the pharyngeal soft tissue, macroglossia and mucosal thickening of the upper airways and bronchi are the main causes of the obstructive form. Some studies have reported a correlation between the severity of SAS and both disease activity and GH/IGF-I levels (213,214), however others have

not found this correlation (207,210). Following treatment, SAS can persist in a relative high percentage of patients with inactive disease (210).

Given the association between SAS and ischaemic heart disease, impaired glucose metabolism and hypertension, there is a possibility that SAS may contribute to the increased vascular mortality in acromegaly, although further studies are required to prove this. In the meantime, it is important to manage SAS effectively, taking into account the fact that the condition may persist even after acromegaly has been successfully treated.

The impact of tumour size on mortality in acromegaly was also considered in this study. Neither tumour size (macroadenoma *versus* microadenoma) nor tumour extension (beyond the sella compared to within the sella) had any impact on survival.

In conclusion, this study highlights the potential harmful effect of external radiotherapy, supporting the view that medical therapy may now be a more appropriate second line treatment in many patients. A diagnosis of diabetes mellitus is a poor prognostic marker in patients with acromegaly, but further studies are required to determine whether diabetes mellitus is primarily responsible for the poor outcome in these patients or whether glucose intolerance is a surrogate marker for patients with higher GH levels, who are known to have a poor prognosis independently of any other factors.

5. MEDICAL THERAPY FOR ACROMEGALY

Despite surgery, around 50% of patients fail to achieve biochemical targets shown to correlate with normalisation of mortality rates. Radiotherapy is effective in controlling tumour growth and GH secretion, but as discussed in Chapter 4, a number of safety issues have been raised with this treatment modality. Medical therapy, therefore, has traditionally played an important role as adjuvant therapy in patients who fail to achieve control with surgery, or while waiting for radiotherapy to take effect. Furthermore, medical therapy is increasingly being used as primary therapy. In this chapter, I explore the role of medical therapy in the management of acromegaly focussing specifically on somatostatin analogue therapy.

There are currently three drug classes available for the treatment of acromegaly; dopaminergic agonists, somatostatin analogues, and GH receptor (GHR) antagonists.

Dopamine agonists are the only oral medication available for the treatment of acromegaly, but their efficacy is limited (63,64), and use of dopamine agonists has largely been superseded by the introduction of somatostatin analogues, which exert their biological effects by activating somatostatin receptors (predominantly sub-receptor types 2 and 5) in the pituitary (66). The native peptide somatostatin exists in two biologically active forms, somatostatin-14 and somatostatin-28, and is widely distributed in the central nervous system and peripheral tissues (215). It exerts its biological effects by activating somatostatin receptors. *In vitro*, native somatostatin has an inhibitory effect on GH secretion in many GH-secreting tumours, and this has led to the development of somatostatin analogues for use in the treatment of acromegaly (216). The two somatostatin analogues available for clinical use are the cyclic octapeptides

octreotide (Dphe-cys-phe-Dtrp-lys-thr-cys-thr-ol) and lanreotide (Dnal-cys-tyr-Dtrp-lys-val-cys-thr) (66).

There are five distinct somatostatin receptors, types 1–5 (217). They are all G protein-coupled receptors, but differ in their regulation, action, tissue distribution and binding affinity to somatostatin analogues (217). Endogenous somatostatin suppression of GH occurs via receptor subtypes 2 and 5, and these are also the predominant types of somatostatin receptors found in GH-secreting pituitary tumours (218).

In contrast to native somatostatin which binds to all 5 receptors, octreotide and lanreotide have greatest affinity for receptor subtypes 2 and 5, with their affinity for subtype 2 being about 10-fold higher than for subtype 5 (215).

The first preparation of somatostatin analogue available for clinical use was subcutaneously-administered octreotide. Since the mid 1990s, octreotide has been available as a long-acting release preparation, octreotide LAR, with octreotide enclosed in microspheres of a slowly biodegrading polymer that allows for prolonged release of the drug (219). After the injection of octreotide LAR, octreotide levels rise briefly, corresponding to release of octreotide from the surface of the microspheres. Octreotide levels then fall and begin to rise again about 7–14 days after the injection and remain elevated for an average of 34 days (67). Lanreotide is also available in a slow release (SR) preparation, with lanreotide encapsulated in microspheres of a biodegradable polymer. After injection of lanreotide SR, lanreotide levels remain elevated for around 11 days (220). Lanreotide Autogel is a newer, viscous, supersaturated, aqueous solution of lanreotide, available in a prefilled syringe and administered by deep subcutaneous injection every 28 days. Lanreotide Autogel has a first order kinetic profile with reduced burst release and a more consistent drug release over the dose period. Maximal serum concentrations are reached

1–2 days after injection in healthy subjects and the serum half-life is 25.5 days (221,222). These long-acting preparations have been shown to be both effective and safe, suppressing GH levels to less than 2-2.5μg/L and normalising serum IGF-I levels in 50-70% of cases (66-69). In addition, tumour shrinkage by 20-50% has been documented in around 30% of patients pre-selected for octreotide responsiveness (66,70-73).

Pegvisomant is a genetically engineered GH receptor antagonist that inhibits GH action rather than secretion. It exerts its biological actions by preventing functional dimerisation of the GH receptor (78). Clinical studies have demonstrated that pegvisomant is remarkably effective, improving clinical symptoms and signs and resulting in IGF-I normalisation in over 90% of patients (79,80). More recently, data collated from the German Acrostudy database during 2 years of treatment with pegvisomant in a clinical practice setting have shown lower rates of control, with 64% of patients achieving a serum IGF-I within the reference range at 6 months and 74% at 24 months (223). The drug appears to be safe and well tolerated. Despite normalised IGF-I levels, GH levels remain elevated in these patients, albeit with minimal or neutralised bioactivity because of receptor blockade. Concerns surrounding tumour growth, deranged liver function and the clinical impact of antibody formation are being addressed as experience with the use of this drug grows (82).

In this section I compare the efficacy of somatostatin analogue treatment when used as primary therapy or as adjunctive therapy, report on long-term efficacy and safety of somatostatin analogues for treatment of acromegaly as primary or adjuvant therapy and discuss factors influencing the decision to treat patients with acromegaly with primary medical therapy.

5.1 Efficacy of somatostatin analogue treatment when used as primary therapy or as adjunctive therapy

5.1.1 Introduction

Current treatments for acromegaly attempt to control the disease by reducing growth hormone secretion from the pituitary tumour either by surgery, radiotherapy or medication. Most clinicians would argue that transsphenoidal surgery remains the most cost-effective and rapid initial treatment of choice for the majority of patients with acromegaly. The question then arises as to how effective this is at achieving 'target' or 'safe' GH and IGF-I levels. Large tumour size together with the degree of extension and high preoperative serum GH levels are suggested to be major determinants of surgical failure (28,224). Application of criteria for successful or 'safe' surgery to various different centres reveals very different outcomes; successful surgical outcome relates closely to the skill and experience of the operator. Transsphenoidal surgery should be undertaken by an experienced operator in an expert unit and under these circumstances, if rigorous criteria are used for the interpretation of surgical results (mean GH <5 mU/L (<2 or 2.5 μg/L), GH nadir after OGTT <1 μg/L, normal IGF-I), between 80-90% of patients with microadenomas but less than 50% of patients with macroadenomas achieve satisfactory biochemical control (32,57,59,87,224). Microadenomas comprise only 20-30% of newly-diagnosed patients with acromegaly (225). It is clear therefore that many patients who undergo initial surgery will require additional therapy, such as radiation and/or medical therapy, to alleviate potentially disabling symptoms, and to control GH and IGF-I levels.

Somatostatin analogue therapy has been proven to be an effective adjunctive therapy in patients that have already been treated with surgery and/or radiotherapy. In addition, several studies in which newly diagnosed patients with acromegaly were given subcutaneous (SC) octreotide preoperatively for short periods of time before surgery report reductions in GH and IGF-I levels and variable effects on tumour size. In the study from Newman *et al*. (74), SC octreotide administration in previously untreated (*de novo*) patients resulted in suppression of GH and IGF-I levels to a similar extent to that observed in patients who received octreotide treatment after surgery. These observations led the authors to conclude that if the possibility of surgical cure is low, and if there is no visual compromise, then medical treatment with octreotide alone should be as effective biochemically and clinically as the combination of surgery followed by octreotide, and may be a reasonable primary therapeutic modality.

A number of studies of depot somatostatin analogues with prolonged release have compared outcomes in *de novo* patients and those who have previously been treated with pituitary surgery or radiotherapy (71,226). Major limitations of these reports remain, in particular the absence of data at diagnosis of acromegaly rather than at entry to the study. The recommendation for tumour debulking even in the anticipation of active disease remaining after surgery is based on the premise that effective treatment with any modality is related to pre-treatment GH values. In this study I attempted to address some of these issues, in particular examining response to treatment in relation to diagnostic baseline GH and IGF-I data. I retrospectively analysed the GH and IGF-I data from a large multicentre European study (69) in which patients' biochemical response to treatment with Octreotide LAR (Novartis, Basle) as primary or adjuvant therapy was evaluated after 12 months of treatment. In the original study, the reported baseline GH and IGF-I concentrations were measured prior to starting Octreotide LAR, but at a time when patients were

on sc octreotide. Thus the effects of previous surgery and/or radiotherapy were not taken into account. In this re-analysis I used GH and IGF-I levels at diagnosis as baseline values to eliminate this element of pre-selection bias and to assess the effects of previous therapeutic interventions on response.

5.1.2 Methods

The study was set up as a prospective, open-label, multicentre study. The original study cohort consisted of one hundred and fifty one patients with acromegaly (74 female) with a mean age of 49.9 years (range 24-81 years) from 40 European centres in 8 countries. The diagnosis of acromegaly was based on raised serum GH concentrations, failure of serum GH to suppress to <2 μg/L after a 75g oral glucose tolerance test, elevated age-matched serum IGF-I levels and clinical features of acromegaly. To be eligible for the study, patients had to be on a stable dose of Octreotide SC (0.1-0.5 mg twice or three times daily) as primary or adjuvant therapy for at least 4 weeks (range 4-250 weeks) prior to screening.

After written informed consent was obtained from each patient, screening serum IGF-I concentrations, 4-hour GH profile, standard safety laboratory tests and clinical examinations were performed. Two weeks later (day 1 of study) all patients with a mean 4-hour serum GH concentration below 10 μg/L on SC treatment underwent a further 4-hour GH profile, IGF-I assessment and the full clinical and safety evaluation. Thereafter the first 20 mg dose of Octreotide LAR was administered intramuscularly (IM) into the gluteal muscle. Patients received 6 injections of Octreotide LAR at 4-weekly intervals, with an option to continue for an additional 6 injections in an extension of the study. The first three injections consisted of the 20 mg dose,

while the last 9 injections consisted of either 10, 20 or 30 mg doses, the dose being titrated according to the mean 4-hour serum GH concentrations recorded after the second 20 mg dose; 10 mg if the mean 4-hour GH concentration was below 1 μg/L, 20 mg if the mean GH concentration was between 1.1 and 5 μg/L and 30 mg if the mean GH concentration was above 5 μg/L. A clinical evaluation of signs and symptoms of acromegaly was made at each visit.

The efficacy criteria were the mean 4-hour serum GH concentrations as primary criterion, and the serum IGF-I concentrations and symptoms of acromegaly as secondary criteria. The mean of the 4-hour GH profiles evaluated at days -14 and 1 were taken as baseline mean GH serum concentrations in the original study.

Samples for IGF-I assay were collected at 8:00 and 9:00 on the same days that samples for GH profiles were assessed and measurements were carried out in one central laboratory. Serum GH concentration was assessed by a double monoclonal antibody technique (Delfia Kit, Wallac OY, Turku, Finland), serum IGF-I concentrations were measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA) after an ethanol-acid extraction.

Retrospectively, I contacted all centres involved in the original study and asked them, wherever possible, to provide the patients' serum GH and IGF-I levels at diagnosis. I reanalysed the study data using these levels as baseline values to eliminate any pre-selection bias caused by previous surgery and/or radiotherapy. Only data from patients who had completed the second part of the study (48 weeks) were considered for re-analysis. Comprehensive data on GH levels at diagnosis were available in 91 of the 151 patients who participated in the original study. Further information on serum IGF-I levels at diagnosis was available in 67 of the 91 patients.

5.1.3 Statistical analysis

Measurements of GH and IGF-I are expressed as the mean ± SEM and the Mann-Whitney test was used to assess the effect of treatment on biochemical parameters. A value of $p<0.05$ was considered statistically significant.

5.1.4 Results

GH data at diagnosis were available in 91 patients (49 female) with a mean age of 50 years (25-81) (Table 5.1). 34 patients were receiving Octreotide LAR as primary medical therapy, having received no previous definitive treatment, while 29 had previously undergone surgery, 23 had been treated with surgery and radiotherapy and 5 had received radiotherapy alone.

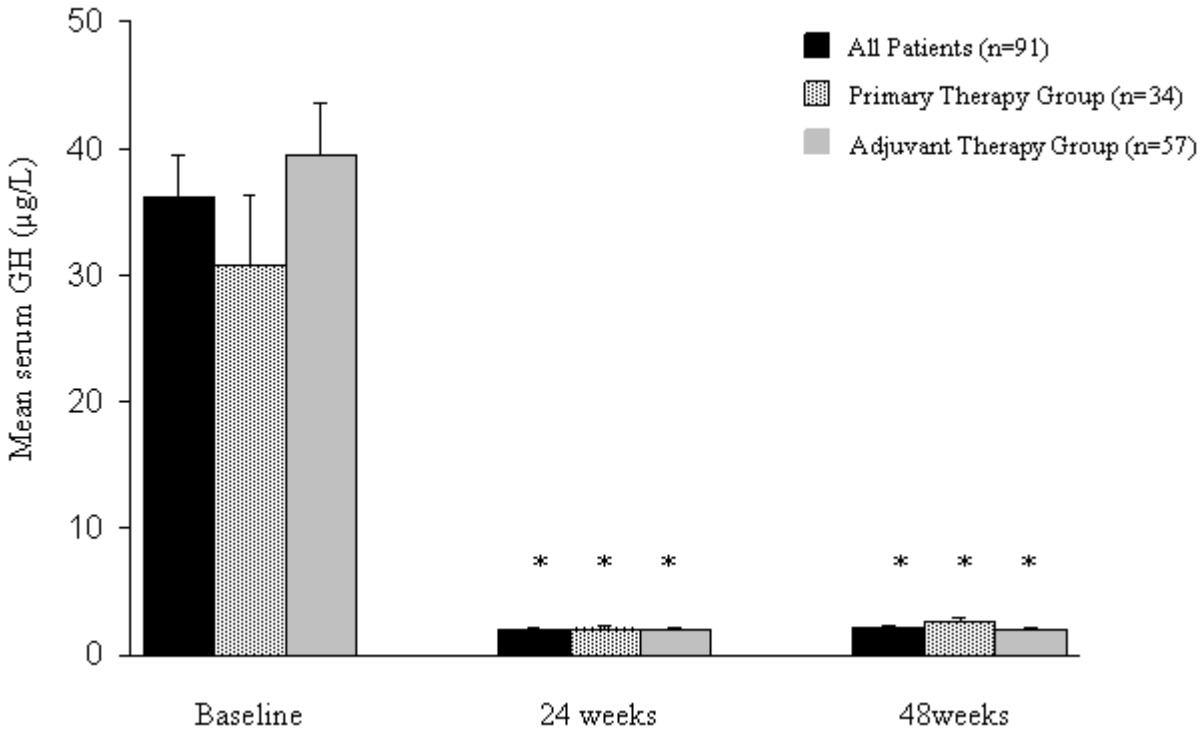
	All	**Primary Therapy**	**Adjuvant Therapy**
n (female)	91 (49)	34 (17)	57 (32)
Mean age in years (range)	50 (25-81)	53 (32-81)	50 (25-73)
Macroadenomas (n)	56	17	39
Microadenomas (n)	21	13	8

Table 5.1: Patient characteristics of 91 patients in whom GH data at diagnosis were available

Individual baseline serum GH levels were obtained by using the mean of 5 values measured during a 2-hour 75 g OGTT performed prior to any form of treatment being commenced for acromegaly.

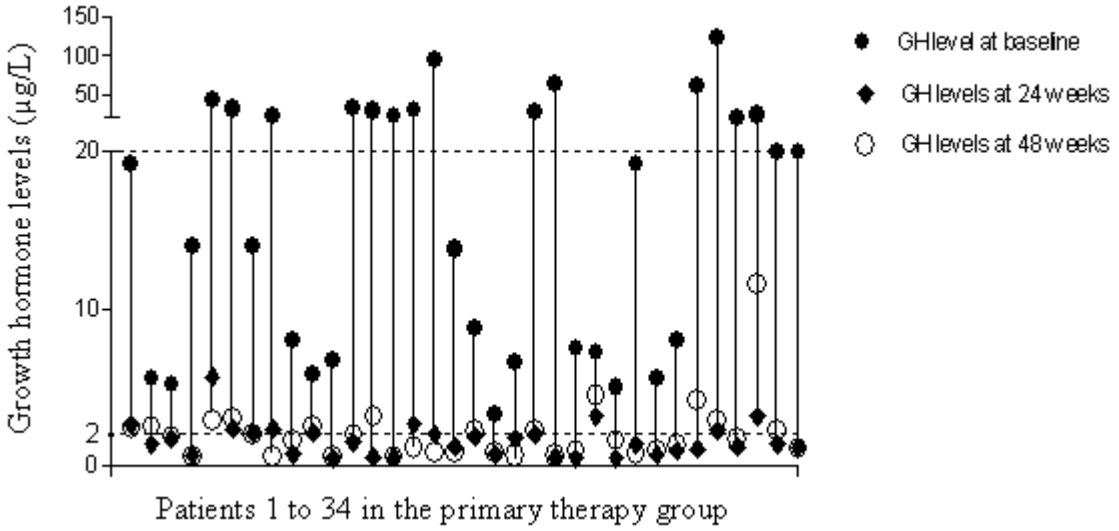
Mean serum GH in the whole group fell from 36.2 ± 3.3 μg/L at diagnosis to 2.0 ± 0.2 μg/L ($p<0.0001$) following 24 weeks of treatment with Octreotide LAR and 2.2 ± 0.2 μg/L ($p<0.0001$ *versus* baseline) following 48 weeks of treatment (Figure 5.1). Using a serum GH <2 μg/L, 75% of patients achieved "safe" GH levels at 24 weeks, and 67% at 48 weeks. There was no statistically significant difference in GH concentrations between the primary and adjuvant therapy groups at diagnosis or following treatment with Octreotide LAR. In the primary therapy group mean serum GH fell from 30.7 ± 5.7 μg/L at diagnosis to 2.1 ± 0.2 μg/L ($p<0.0001$) following 24 weeks of treatment and 2.6 ± 0.4 μg/L ($p<0.0001$ *versus* baseline) following 48 weeks of treatment with Octreotide LAR, while in the adjuvant therapy group mean serum GH was 39.5 ± 4.1 μg/L at diagnosis, falling to 1.9 ± 0.2 μg/L ($p<0.0001$) and 2.0 ± 0.2 μg/L ($p<0.0001$ *versus* baseline) after 24 and 48 weeks of treatment respectively. 62% of patients in the primary therapy group and 70% in the adjuvant therapy group achieved "remission" using GH criteria (as defined above) following 48 weeks of treatment. There was no statistically significant difference in GH levels between the primary and adjuvant therapy groups at diagnosis, pre-Octreotide LAR or after 48 weeks of treatment. 16 patients in the primary therapy group had pre-treatment GH levels > 20 μg/L. Of these, 7 (44%) achieved a GH level less than 2 μg/L (Figure 5.2) and 9 (56%) had IGF-I normalisation. In the adjuvant therapy group, 35 patients had pre-treatment GH levels greater than 20 μg/L of whom 23 (66%) achieved a GH level less than 2 μg/L (Figure 5.3) and 27 (77%) had IGF-I normalisation.

Figure 5.1



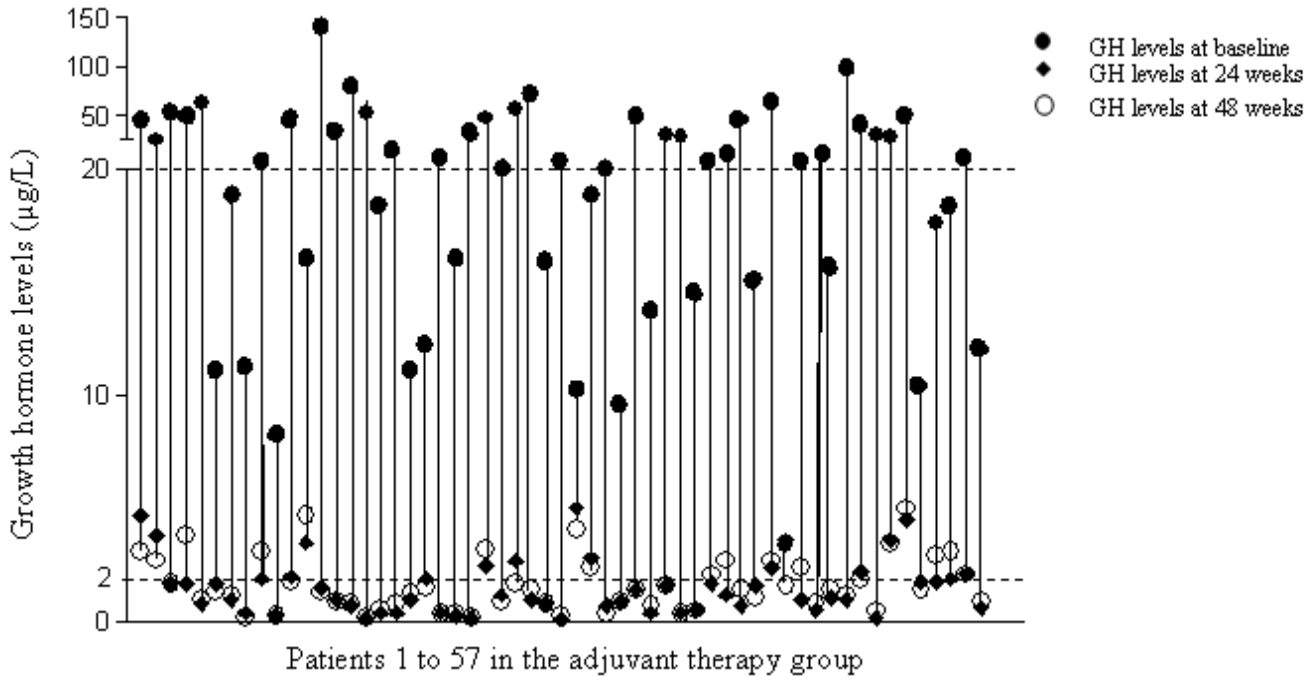
Mean serum GH concentrations (± SEM) at diagnosis, after 24 weeks of treatment ($p < 0.0001$ versus baseline) and after 48 weeks of treatment with Octreotide LAR*

Figure 5.2



Mean serum GH levels at baseline, following 24 weeks of treatment and following 48 weeks of treatment in 34 patients with acromegaly treated with Octreotide LAR as primary medical therapy.

Figure 5.3



Mean serum GH levels at baseline, following 24 weeks of treatment and following 48 weeks of treatment in 57 patients with acromegaly treated with Octreotide LAR as adjuvant therapy.

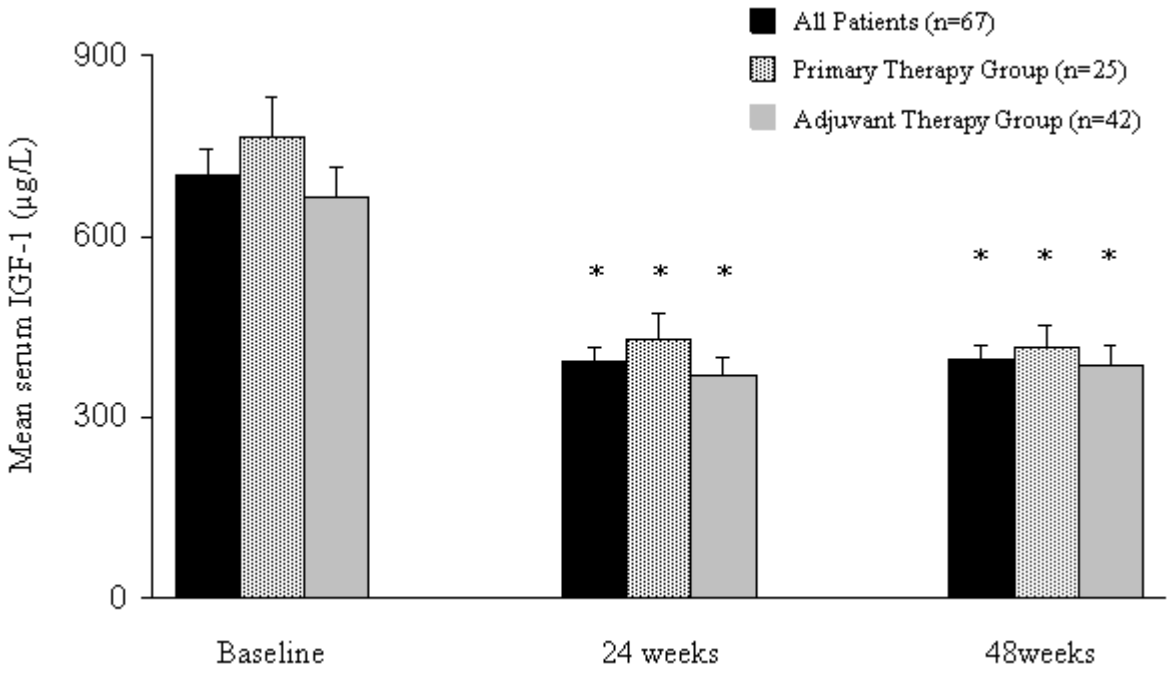
Additional information on serum IGF-I levels at diagnosis was available in 67 patients (33 female) with a mean age of 50 years (28-81). Patient characteristics are demonstrated in Table 5.2.

	All	**Primary Therapy**	**Adjuvant Therapy**
n (female)	67 (33)	25 (12)	42 (21)
Mean age in years (range)	50 (28-81)	52 (32-81)	49 (28-73)
Macroadenomas (n)	41	12	29
Microadenomas (n)	15	9	6

Table 5.2: Patient characteristics of 67 patients in whom IGF-I data at diagnosis were available

25 patients were receiving Octreotide LAR as primary therapy and 42 as adjuvant therapy. In the whole group mean serum IGF-I was 703 ± 40 μg/L at diagnosis, falling to 392 ± 24 μg/L ($p<0.0001$) and 395 ± 25 μg/L ($p<0.0001$ *versus* baseline) following 24 and 48 weeks of treatment with Octreotide LAR respectively (Figure 5.4). There was no statistically significant difference in IGF-I concentrations between the primary and adjuvant therapy groups at diagnosis or following treatment with Octreotide LAR.

Figure 5.4



Mean serum IGF-I concentrations (± SEM) at diagnosis, after 24 weeks of treatment ($p < 0.0001$ versus baseline) and after 48 weeks of treatment with Octreotide LAR*

In the primary therapy group, mean serum IGF-I fell from 764 ± 68 μg/L at diagnosis to 430 ± 41 μg/L ($p<0.0001$) following 24 weeks of treatment and to 414 ± 37 μg/L ($p<0.0001$ *versus* baseline) following 48 weeks of treatment with Octreotide LAR, while in the adjuvant therapy group mean serum IGF-I was 666 ± 50 μg/L at diagnosis, falling to 369 ± 30 μg/L ($p<0.0001$) and 384 ± 33 μg/L ($p<0.0001$ *versus* baseline) following 24 and 48 weeks of treatment with Octreotide LAR respectively. Overall 72% of patients achieved normal age-matched serum IGF-I levels after 48 weeks of treatment, 64% in the primary therapy group and 76% in the adjuvant therapy group. There was no statistically significant difference in IGF-I levels between the primary and adjuvant therapy groups at diagnosis, pre- Octreotide LAR or after 48 weeks of treatment.

5.1.5 Discussion

This retrospective analysis of data from a large multicentre European study demonstrates that in a group of patients with similar diagnostic GH and IGF-I levels, Octreotide LAR was equally effective as primary therapy as it was as adjunctive therapy in patients with acromegaly previously treated with surgery and/or radiotherapy. There were no statistically significant differences in GH and IGF-I levels between the primary and adjuvant therapy groups at diagnosis, pre-Octreotide LAR and after 12 months of treatment. The study indicates that the pre-treatment GH and/or IGF-I values were not major factors in determining whether biochemical responsiveness was similar in the two groups, thus eliminating one element of pre-selection bias.

Several previous studies have included newly diagnosed patients with acromegaly who had not undergone pituitary surgery or irradiation, and were treated for variable periods of time with subcutaneous octreotide or depot somatostatin analogues preparations. In a study reported by Newman and colleagues (74), with the objective of determining whether primary therapy for acromegaly with Octreotide was effective long term, the effects of subcutaneous Octreotide (up to 5 years) in 26 previously untreated patients with acromegaly (primary treatment group) were compared with those in 81 patients who had initially been treated with surgery and/or pituitary irradiation (adjuvant treatment group). These patients were part of a multicentre study that took place between 1989 and 1995, and 2 previous reports have been published on the results in the group as a whole (227,228). Patients whose GH levels fell to at least 2 SD below the baseline mean GH were considered responders. There was no significant difference in the percentage of responders in the primary and adjuvant treatment groups (70% *versus* 61%). Improvement in symptoms of headache, increased perspiration, fatigue, and joint pain was similar in the two groups. These findings are also supported by the results of a recent study assessing the efficacy of Lanreotide Autogel in somatostatin analogue-naive patients with acromegaly (229). Fifty-one patients, 39 newly diagnosed (*de novo*) and 12 who had previously undergone unsuccessful surgery, were studied. All patients initially received 120 mg of lanreotide Autogel every 8 weeks for 24 weeks. Subsequently the time interval between injections was adjusted to 4 or 6 weeks, or remained at 8 weeks, depending on biochemical response. GH < 2.5 μg/L was achieved in 32 patients (63%), with no difference between the *de novo* and post-operative patients (72% *versus* 50%, p=0.48). IGF-I levels normalised in 19 patients (37%), again with no difference between the *de novo* and post-operative patients (33% *versus* 50%, p=0.48).

In this study the pre-treatment GH and/or IGF-I values were not major factors in determining biochemical responsiveness to medical treatment in either the primary or adjuvant therapy groups. Not all studies support the notion that pre-treatment GH levels are not an important factor in predicting response to somatostatin analogues. In a multicentre trial to determine the efficacy and safety of the somatostatin analogue octreotide acetate in 103 patients (228), when the patients were grouped according to pre-treatment GH levels, those with higher initial GH concentrations were less likely to normalise IGF-I during treatment, and indeed a significant minority showed sub-optimal reductions in GH and IGF-I. More recently, the United Kingdom Primary Octreotide Therapy Study Group reported on 27 patients with newly diagnosed acromegaly who were treated with sc octreotide and Octreotide LAR as primary medical therapy for up to 48 weeks (72). At the end of the study, of patients with pre-treatment GH levels greater than 50 mU/L (20 μg/L), only 1 achieved a GH level less than 5mU/L (2 μg/L) and none achieved IGF-I normalisation. By contrast in our analysis, of the 16 patients in the primary therapy group with pre-treatment GH levels greater than 20 μg/L, 7 (44%) achieved a GH level less than 2 μg/L (Figure 5.2) and 9 (56%) had IGF-I normalisation. The reason for this discrepancy is not entirely clear, although it is worth noting that fewer patients in our primary therapy group had macroadenomas (50%) than in the United Kingdom Primary Octreotide Therapy Study Group (74%).

To assess the impact of tumour size on outcome, we performed a more detailed analysis of the data comparing outcomes in patients with macroadenomas and microadenomas (Table 5.3). These data show that the mean GH levels at diagnosis were higher in the macroadenoma group (30.5 ± 3.8 *versus* 19.1 ± 3.3 μg/L, $p = 0.072$) but did not attain statistical significance. The

proportion of patients achieving normalised GH and IGF-I levels were virtually identical in each group, thus indicating that in this cohort, tumour size was not a major determinant of outcome.

	Macroadenomas	**Microadenomas**	
n (female)	56 (28)	21 (14)	
Primary Medical Therapy	17	13	
GH at diagnosis ± SEM (μg/L)	30.5 ± 3.8	19.1 ± 3.3	p = 0.072
GH at 48 weeks ± SEM (μg/L)	1.7 ± 0.2	1.9 ± 0.3	p = 0.20
GH < 2μg/L	66%	62%	
IGF-I normal	68%	62%	

Table 5.3: Effect of tumour size on outcome showing mean GH concentrations at diagnosis and following 48 weeks of treatment

A number of questions remain after analysis of these data. Patients entering studies were not randomised to primary therapy with somatostatin analogue *versus* surgery; in this study, as in others, the entry criteria ensured that only patients with disease responding to somatostatin analogue therapy were included in the study. Another limitation arises from the fact that data for re-analysis were available in only 91 of the 151 subjects reported on in the original study. In addition any group of previously treated patients was obviously selected to exclude patients who were cured by transsphenoidal surgery. The effect of somatostatin analogue treatment on tumour size has been the focus of a number of recent studies (71-73) and a further drawback arises from

the fact that change in tumour size was not formally assessed during the original study. However, patients continued to undergo pituitary imaging as part of their routine follow up and it is important to emphasise that no patients were withdrawn from the study due to tumour enlargement.

Although a number of retrospective studies suggest a beneficial effect of partial surgical reduction of tumour mass on subsequent responsiveness to medical treatment and outcome (230,231), the case far from proven. Petrossians *et al*. examined GH and IGF-I response to somatostatin analogue therapy before and after surgery in 24 patients with acromegaly caused by large invasive tumours (230). Of the 17 patients who did not achieve GH values < 2 μg/L with preoperative medical therapy, six achieved this target with postoperative somatostatin analogue treatment. Of the 13 patients who did not normalise IGF-I values with preoperative medical therapy, seven achieved normal IGF-I levels with postoperative somatostatin analogue therapy. The magnitude of the decrease in GH with somatostatin analogue therapy following surgical debulking was virtually identical to that seen before surgery. Similarly, Colao *et al*. demonstrated significant benefit of debulking surgery in patients with poor response to somatostatin analogue therapy if tumour volume reduction of at least 75% was achieved (231). In the only prospective study to explore this issue, 26 patients with macroadenomas were treated with lanreotide prior to surgery and then re-assessed off medical treatment at 16 weeks following surgery (232). When lanreotide was reintroduced in 6 patients who did not achieve "safe" GH levels, GH fell to safe levels in 3 of them. Our findings and those from other studies (66,71,74,75) do not corroborate this, suggesting the evidence remains inconclusive. It can be argued that until partial debulking of a GH-secreting tumour is shown to be of specific benefit in a large controlled study, the finding that somatostatin analogue therapy is as effective in previously untreated patients as it is

in previously treated patients indicates that in patients with documented responsiveness to octreotide, primary treatment with a somatatostatin analogue may be just as beneficial as the combination of surgery and/or radiation and medical therapy. If it is unlikely that complete tumour removal will occur (as is the case with many macroadenomas or invasive tumours), and if there is no visual compromise, these data suggest that medical treatment alone should be as effective biochemically and clinically as the combination of surgery followed by somatostatin analogue.

Primary medical therapy for some patients with acromegaly is now a realistic treatment option. Factors that are important in choosing an optimal strategy should include likelihood of surgical success as well as patient preference, coincidental co-morbidity and cost-benefit analyses. Some of these factors will be discussed in greater detail in Section 5.3. Exactly what proportion of patients should be treated with primary medical therapy remains controversial, and until we have a large prospective randomised trial in which all patients are treated medically and non-cured ones are treated again following surgery, it will be difficult to answer these questions conclusively. In the mean time, further evaluation is needed of long-term efficacy of biochemical control, long-term morbidity, mortality and quality of life, effect on tumour size, and side effects of medical therapy. In the next section (Section 5.2), some of these issues are explored.

5.2 Long-term safety and efficacy of depot long-acting somatostatin analogues for the treatment of acromegaly

5.2.1 Introduction

Historically transsphenoidal surgery and/or radiotherapy have been considered the treatment of choice for acromegaly, but despite recent advances in both these forms of treatment, the overall surgical cure rate remains around 60% and radiotherapy may take 5-10 years to lower GH to an "acceptable" level (233). Somatostatin analogues have been used as an adjunct to surgery and/or radiotherapy, but they are increasingly being used as first line therapy in the treatment of acromegaly. Octreotide is a long-acting synthetic somatostatin analogue which has been used to treat acromegaly for over 15 years. Since the mid-1990s, three slow-release depot preparations have been introduced; Sandostatin LAR (Novartis), Lanreotide LA (Ipsen) and Lanreotide Autogel (Ipsen). Previous studies, many of them limited to 12 months of follow-up, have shown them to be both effective and safe, suppressing GH levels to <2 μg/L in 50-65% of cases and normalising serum IGF-I levels in 65% of cases (67-69,71,234,). In the previous chapter, we demonstrated that in patients with documented responsiveness to octreotide, somatostatin analogue therapy is as effective in previously untreated patients as it is in patients previously treated with surgery and/or radiotherapy. Published data on the long-term efficacy and safety of these depot analogues beyond 1 year are scanty (75,235-238), however, and concerns have been raised about tachyphylaxis, tolerability and side effects such as the development of gallstones and glucose intolerance. Against this background, in this part of the project I have analysed outcomes in 22 patients treated with depot somatostatin analogues for up to 89 months.

5.2.2 Patients

22 subjects (16 female) with active acromegaly as determined by the presence of clinical signs and symptoms, failure of GH to suppress below 1 μg/L during a 2-hour 75 g oral glucose tolerance test and raised serum IGF-I levels were selected from a cohort of 31 subjects who had been treated with long-acting somatostatin analogues. Of the nine subjects who were excluded, 6 had been on treatment for less than 12 months, 2 had their treatment stopped following surgery and 1 subject had treatment withdrawn after 10 months due to lack of efficacy. At presentation 12 subjects had macroadenomas and 7 microadenomas. In 2 subjects, 1 with known ectopic growth hormone-releasing hormone secretion secondary to a bronchial carcinoid tumour, pituitary MRI scans were reported as normal, and 1 subject had an empty sella. All subjects were treated either with Sandostatin LAR (Novartis) or Lanreotide LA (Ipsen), and doses were titrated according to biochemical response. In total 18 subjects were treated with Octreotide LAR (2 with a dose of 20 mg every 6 weeks, 11 with 20 mg every 4 weeks and 5 with 30 mg every 4 weeks) and 4 with Lanreotide (3 with 30 mg every 2 weeks and 1 with 30 mg every 10 days). The mean age at the onset of treatment was 52 years (28-69) and the mean duration of treatment was 41 months (12-89). 3 subjects had previously undergone surgery, 2 had received radiotherapy and 7 had been treated with both surgery and radiotherapy. 10 subjects were treated with primary medical therapy, having received no definitive treatment for their acromegaly in the past. The mean pre-treatment GH level was 13.1 ± 3.4 μg/L (range 1.8-67.6) with serum GH < 2 μg/L in 1 subject (5%) and the mean serum IGF-I was 592.2 ± 53.9 μg/L (range 203.3-1074.2) with normal serum IGF-I levels in 4 subjects (18%).

5.2.3 Methods

Assay and measurement details are outlined in Section 2.3. Serum GH levels were measured by an in-house RIA at the Regional Endocrine Laboratory at the University Hospital Birmingham, Selly Oak, as previously described (85). Individual GH levels were obtained by using the mean of 5 values measured during a 2-hour 75 g oral glucose tolerance test. Serum IGF-I was measured using a commercial kit developed by the Nichols Institute (San Juan, California), with acid-ethanol extraction performed to remove IGF binding proteins, as previously described (67). Glucose tolerance was analysed using basal and 2-hour plasma glucose levels from samples taken during a 2-hour 75g oral glucose tolerance test. All subjects had ultrasound scanning of the gallbladder performed by the same radiologist at baseline and at least every 6 months. Pituitary MRI scans were performed at baseline and at regular intervals.

5.2.4 Statistical analysis

Measurements of GH, IGF-I and plasma glucose are expressed as the mean ± SEM and the Wilcoxon matched-pairs signed ranks test was used to assess the effect of treatment on biochemical parameters. A value of $p < 0.05$ was considered statistically significant.

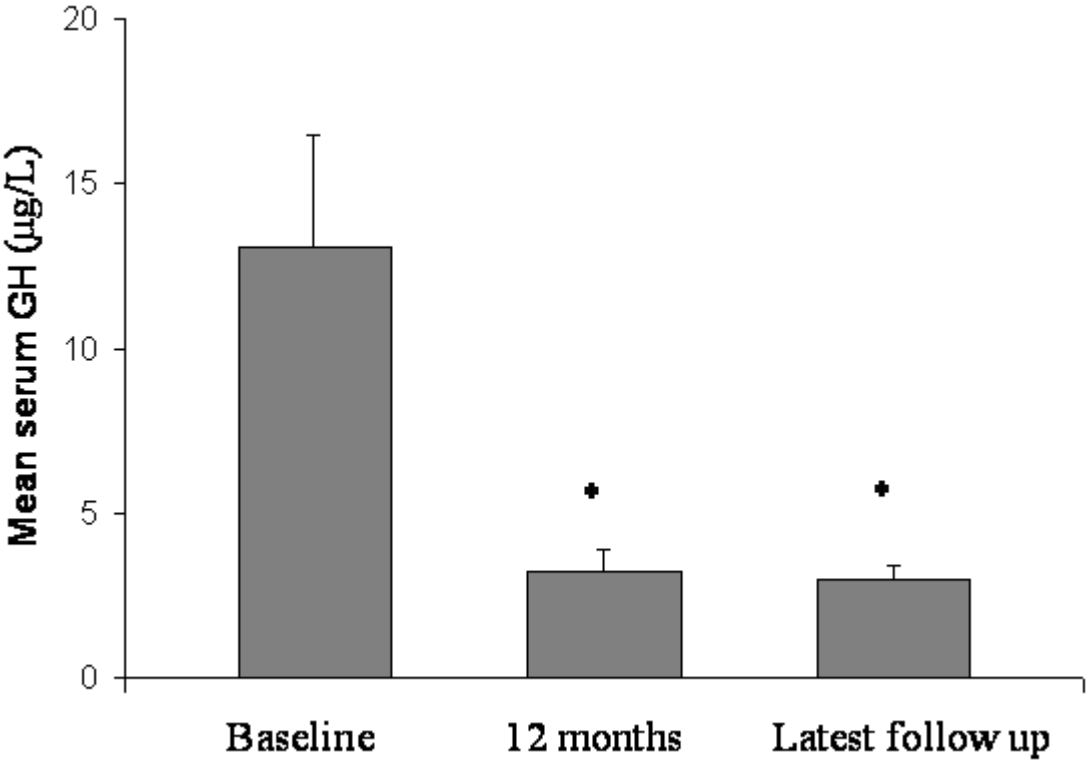
5.2.5 Results

A) Serum GH and IGF-I concentrations

Mean serum GH in the group fell from 13.1 ± 3.4 μg/L at baseline to 3.2 ± 0.7 μg/L ($p < 0.0001$) after 12 months of treatment with a depot long-acting somatostatin analogue (Figure 5.5). This reduction was sustained with long-term treatment, with the mean serum GH concentration at latest follow-up being 3.0 ± 0.4 μg/L ($p < 0.0001$ *versus* baseline). Figure 5.6 shows the course of GH levels in each individual subject with time. Using a serum GH <2 μg/L, 46 % of subjects achieved "safe" levels at 12 months, and 36% at the end of follow-up.

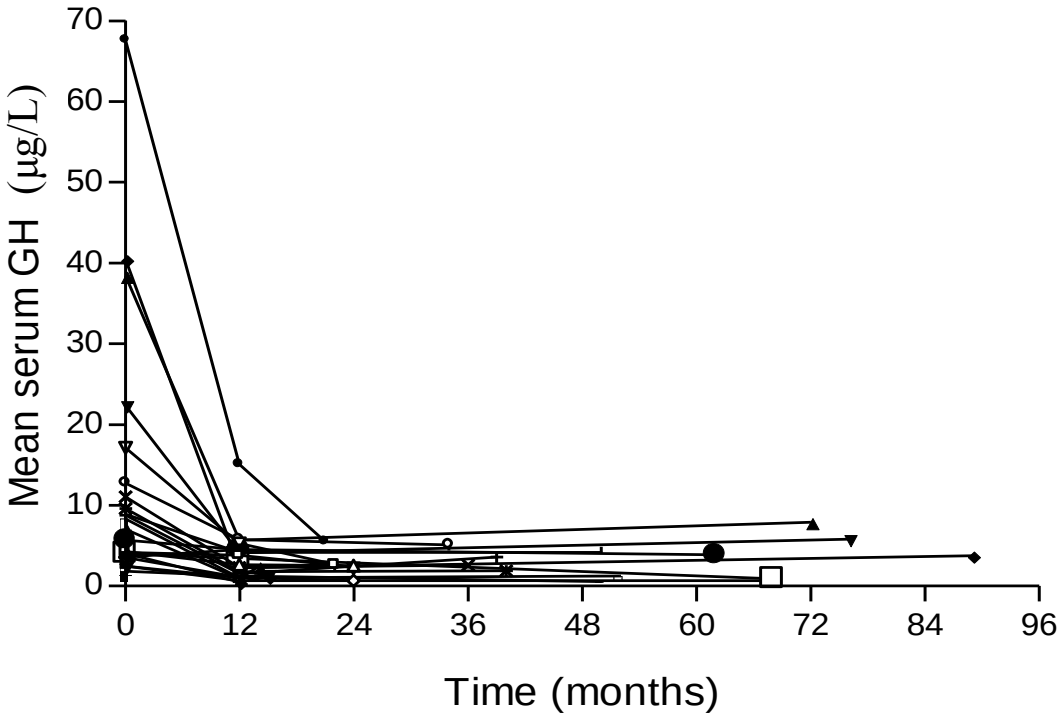
In the whole group the mean serum IGF-I concentration fell from 592.9 ± 53.9 μg/L at baseline to 321.9 ± 33.9 μg/L ($p < 0.001$) after 12 months of treatment and to 278 ± 29.3 at latest follow-up (Figure 5.7). Figure 5.8 shows the course of the serum IGF-I concentration of each individual subject with time. 52% of subjects achieved a normal age- and sex-related IGF-I value 12 months after starting treatment, and 67% at latest follow-up.

Figure 5.5



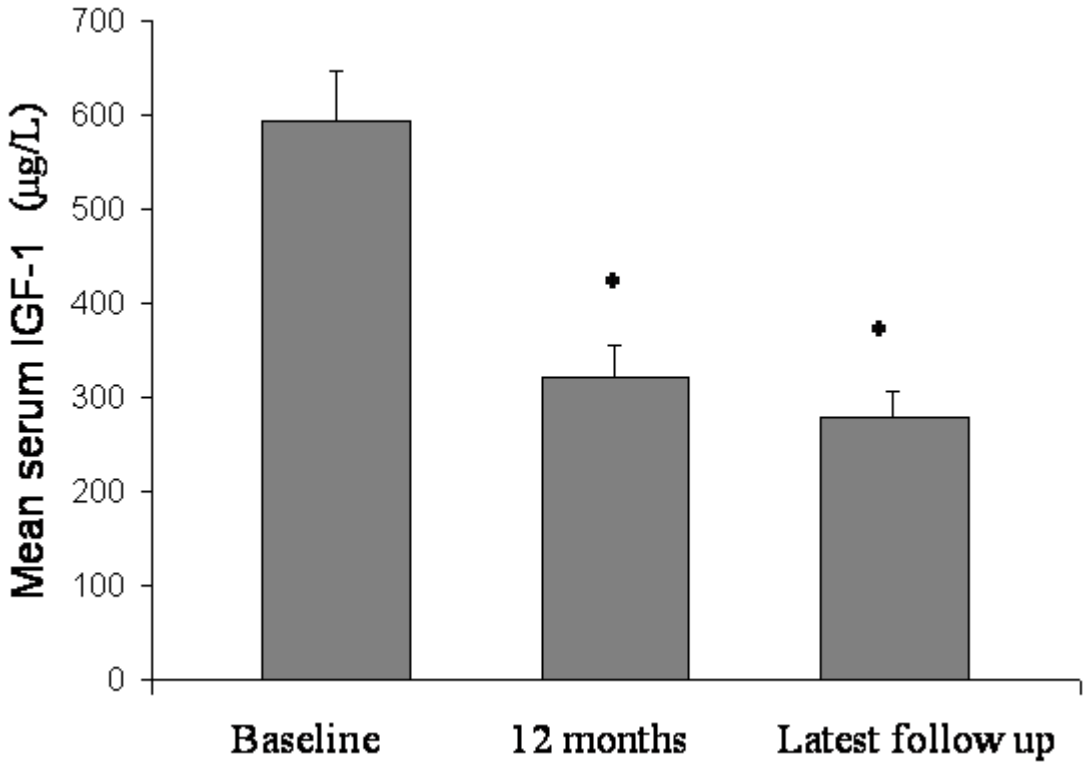
Mean serum GH concentrations (± SEM) at baseline, after 12 months of treatment ($p < 0.0001$ versus baseline) and at latest follow up in 22 patients with acromegaly*

Figure 5.6



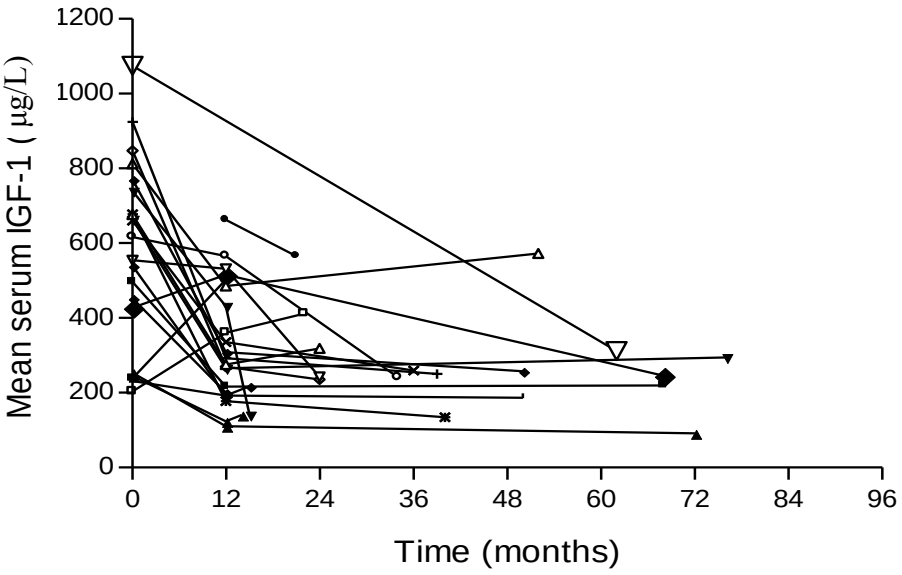
Course of serum GH levels in each individual patient with time; values at baseline, after 12 months of treatment and at latest follow up in 22 patients with acromegaly

Figure 5.7



Mean serum IGF-I concentrations (± SEM) at baseline, after 12 months of treatment ($p < 0.001$ versus baseline) and at latest follow up in 22 patients with acromegaly*

Figure 5.8



Course of serum IGF-I concentrations in each individual patient with time; values at baseline, after 12 months of treatment and at latest follow up in 22 patients with acromegaly

B) Glucose tolerance

Glucose tolerance status was defined using the 1999 WHO criteria for the diagnosis of impaired glucose tolerance and diabetes mellitus (239). Fasting plasma glucose of ≥ 7.0 mmol/l was considered abnormal. Impaired glucose tolerance (IGT) was defined as plasma glucose level between 7.8 and 11.1 mmol/l and diabetes mellitus as plasma glucose level ≥ 11.1 mmol/l measured 2 hours after a 75g oral glucose load. Mean 2-hour OGTT values in the group were similar throughout at 8.5 ± 1.1 mmol/l at baseline, 7.9 ± 0.9 mmol/l after 12 months of treatment and 7.3 ± 0.6 mmol/l at latest follow-up (p = N/S). The corresponding figures for fasting plasma glucose levels were 5.8 ± 0.4 mmol/l, 6.2 ± 0.6 mmol/l and 5.5 ± 0.2 mmol/l respectively. At baseline 6 subjects had impaired glucose tolerance and 2 had diabetes mellitus. After 12 months of treatment, 3 subjects developed impaired glucose tolerance, one of whom went on to develop diabetes mellitus. A further subject developed impaired glucose tolerance at latest follow-up. However, glucose tolerance returned to normal in 4 subjects with impaired glucose tolerance after 12 months of treatment, and in an additional one at latest follow-up.

C) Gallstones

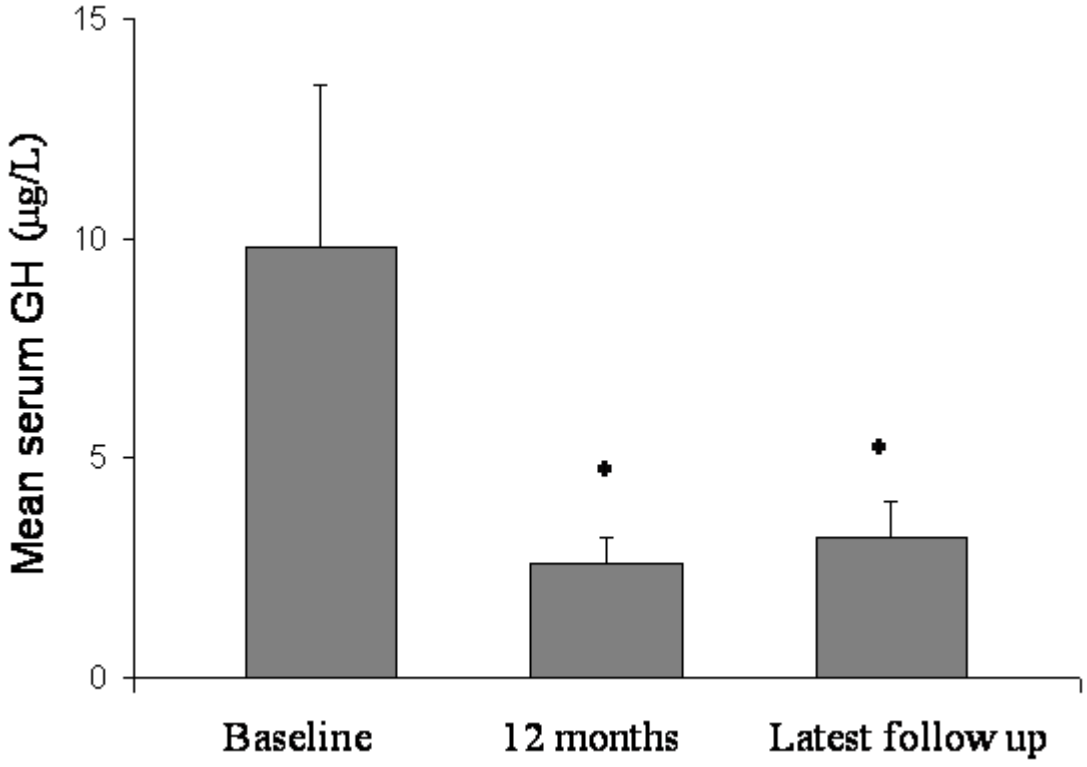
All subjects had serial ultrasound scanning of the gallbladder performed by the same radiologist at baseline and at least every 6 months. One subject was found to have evidence of significant gallbladder wall thickening at baseline and underwent a laparoscopic cholecystectomy. 5 subjects (23%) developed new gallstones during the study period and one developed gallbladder sludge. In 4 subjects, gallstones developed 24-40 months after commencing treatment with long-acting somatostatin analogues and persisted throughout the study period, while in the remaining 1 subject gallstones were identified 2 months into the study period and steadily increased in

number. The gallbladder sludge was identified 34 months after commencing treatment and did not progress to gallstones during the study period (52 months). Only one of these subjects developed symptoms related to cholelithiasis, requiring referral to a gastroenterologist.

D) Primary medical treatment

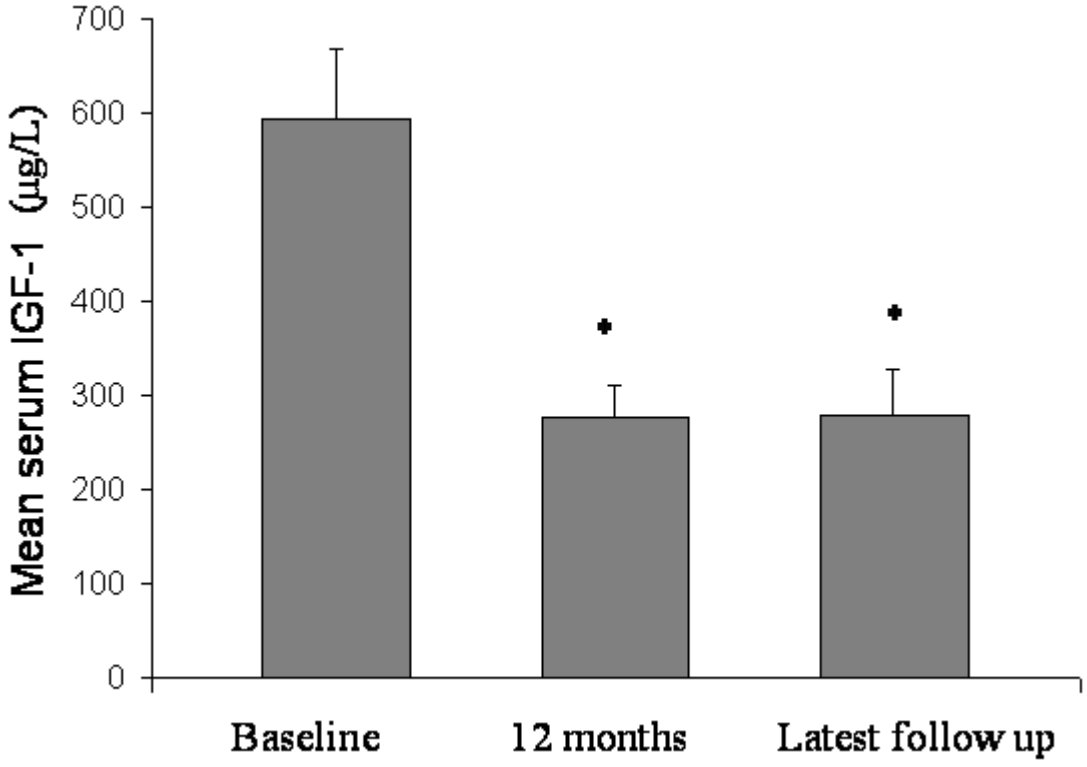
Of the 22 subjects, 10 (8 female) received depot long-acting somatostatin analogues as primary therapy, having had no definitive treatment for their acromegaly in the past. The mean age at the onset of treatment was 58 years (45-69) and the mean duration of treatment was 40 months (12-76). 6 subjects had microadenomas, one a macroadenoma without suprasellar extension and one an empty sella. Pituitary MRI scans were reported as normal in 2 subjects. Mean serum GH in this group was 9.8 ± 3.7 μg/L at baseline, falling to 2.6 ± 0.6 μg/L ($p < 0.01$) after 12 months of treatment and 3.2 ± 0.8 μg/L at latest follow-up (Figure 5.9). 50% of subjects achieved "remission" using GH criteria (as defined above) at 12 months, and 40% at latest follow-up. Mean serum IGF-I was 592.9 ± 74.7 μg/L at baseline, falling to 276.4 ± 33.9 μg/L ($p = 0.001$) and 279.5 ± 47.7 μg/L at 12 months and at latest follow-up respectively (Figure 5.10). 50% of subjects achieved normal age- and sex-matched IGF-I values at 12 months and 60% at latest follow-up. Mean 2-hour OGTT values were similar at baseline (8.9 ± 1.9 mmol/l), 12 months (9.1 ± 1.9 mmol/l) and latest follow-up (7.8 ± 1.0 mmol/l). 2 subjects developed impaired glucose tolerance after 12 months of treatment, one of whom went on to develop diabetes mellitus.

Figure 5.9



Mean serum GH concentrations (± SEM) at baseline, after 12 months of treatment ($p < 0.01$ versus baseline) and at latest follow up in 10 previously untreated patients with acromegaly (primary therapy group)*

Figure 5.10



Mean serum IGF-I concentrations (± SEM) at baseline, after 12 months of treatment (p = 0.001 versus baseline) and at latest follow up in 10 previously untreated patients with acromegaly (primary therapy group)*

At latest follow-up, glucose tolerance had returned to normal in 2 subjects with impaired glucose tolerance at baseline. During the study period one subject developed new gallstones (10%) and one developed gallbladder sludge.

5.2.6 Discussion

The primary aims of therapy in acromegaly should be to reverse the symptoms and signs of the disease, treat the underlying cause, prevent disease recurrence and improve long term survival (240). Lowering GH concentrations rapidly achieves symptomatic relief and improves patient wellbeing. A universally accepted definition of a "cure" remains elusive, but there is now evidence that achieving GH levels of < 2-2.5 μg/L is associated with near normal life expectancy (142), making this the aim of therapy wherever possible. Surgery via the transsphenoidal route with or without adjuvant radiotherapy is still considered the treatment of choice, but despite recent advances in both these forms of treatment and the fact that the surgical cure rate for microadenomas may approach 90% in the hands of an experienced pituitary surgeon, the overall surgical cure rate remains around 60% (57) and radiotherapy may take 5-10 years to lower GH to an "acceptable" level (233). Radiotherapy has the additional disadvantage of failing to normalise GH secretion pulsatility so that serum IGF-I levels remain raised (241). Furthermore, such therapies are associated with a high incidence of hypopituitarism, which itself is associated with excess mortality (134,179). These concerns have led to a re-appraisal of medical therapy for acromegaly, not just as an adjunct to surgery and radiotherapy, but for use as first line primary therapy.

Octreotide, a long-acting synthetic somatostatin analogue with a half-life of 80-100 minutes, was first synthesised in 1982 (242) and has been used to treat acromegaly for 2 decades. Administered as a subcutaneous injection three times daily, doses of 100-1500 μg per day have been shown to effectively suppress GH levels to < 4 μg/L in 22-45% of cases and normalise IGF-I levels in 50% of cases (243,244). The impact of frequent daily injections on patient compliance and quality of life and the mounting evidence that continuous octreotide infusions are more effective than subcutaneous regimens (245) provided the impetus for the development of depot long-acting preparations. Since the mid-1990s, three slow-release depot preparations, Sandostatin LAR (Novartis), Lanreotide LA (Ipsen) and Lanreotide Autogel (Ipsen), have been introduced with the aim of abolishing the need for multiple daily injections and improving patient compliance. Previous studies, some of them large multicentre trials, have shown both analogues to be effective and safe, suppressing GH levels to <2 μg/L in 50-65% of cases and serum IGF-I levels to normal in 65% of cases (67-69,71,234). Published data on the long-term efficacy and safety of these depot analogues beyond 1 year are scanty, however, and concerns have been raised about tachyphylaxis, tolerability and side effects such as the development of gallstones and glucose intolerance.

In this series, I have studied outcomes in patients treated with depot somatostatin-analogue preparations for up to 8 years. I observed a significant reduction in GH and IGF-I levels in the whole group after 12 months of therapy and this was sustained at latest follow-up with no evidence for tachyphylaxis. At 36%, the number of patients achieving “remission” using GH criteria at latest follow-up was lower than in previous studies, but 62% achieved normal IGF-I levels, similar to previously reported figures.

The findings of our study have been supported by results of subsequent studies on the safety and efficacy of long-term use of long-acting somatostatin analogues. In 67 consecutive patients with acromegaly (36 women) treated with octreotide LAR for up to 9 years (median follow-up 48 months, range 6-108 months), Cozzi *et al*. demonstrated control of biochemical parameters (GH <2.5 μg/L and IGF-I normalisation) in 68.7 and 70.1% of patients respectively (236). 72% of patients had macroadenomas, and tumour shrinkage occurred in 82.1% by 62 ± 31% (range 0-100%), decreasing overall from 2101 ± 2912 to 1010 ± 2196 mm^3 ($P < 0.0001$). Metabolic parameters did not alter significantly, and gallstones or biliary sludge was documented in 12% of patients. In a further study of 36 patients with acromegaly treated with a long-acting somatostatin analogue as first-line therapy for up to 18 years, GH < 2 μg/L and normal IGF-I were achieved in 70% and 67% of patients respectively (237). 58% of patients achieved both criteria. Mean GH and IGF-I levels continued to decrease over 10 years. 70% of patients had macroadenomas, and tumour shrinkage > 20% occurred in 72%. Again, treatment was well tolerated.

Abnormal glucose metabolism in acromegaly is generally associated with older age, longer disease duration, family history of diabetes, and presence of hypertension (34,43,246). Although most studies have shown no significant effect of somatostatin analogue treatment on overall glucose tolerance, one group reported raised HbA1c levels after 6 months of treatment in 7 of 49 patients with normal HbA1c levels at baseline (247). In our study, overall glucose tolerance did not change, but 4 patients with normal glucose tolerance at baseline developed impaired glucose tolerance at a time when GH levels were either improving or static. These findings are similar to those documented by Colao *et al*. in a recent open, prospective study investigating the impact of primary therapy with somatostatin analogues for 12 months on glucose tolerance in 112 patients with acromegaly (248). In the group as a whole, they demonstrated similar rates of improvement

and deterioration of glucose tolerance; improvement in 11 (9.8%) and worsening in 17 (15.2%) (p = N/S). In the patients with normal glucose tolerance at baseline who did not achieve disease control, there was a small but significant increase in fasting glucose levels after 12 months of somatostatin analogue treatment. In the patients with abnormal glucose metabolism at diagnosis of acromegaly, fasting glucose levels decreased significantly. Worsening of glucose control was more frequent at the beginning of somatostatin analogue treatment in patients with abnormal glucose metabolism at baseline. Worsening of glucose tolerance after 12 months was associated with poor control of acromegaly.

New gallstone formation has been reported in up to 24% of patients treated with depot somatostatin analogues (234,249-251), a figure which is confirmed in this study (23%). In most cases, gallstone formation occurred only after patients had been treated with long-acting somatostatin analogues for at least 24 months, and once identified they remained present for the duration of the study period. In keeping with previous studies, symptomatic cholelithiasis was rare, with only one patient developing symptoms and requiring referral to a gastroenterologist. Although we did not set out to assess change in tumour size, all patients received serial pituitary MRI scans and in no case was an increase in tumour size reported.

Despite the proven efficacy of somatostatin analogue therapy in acromegaly and the limitations of surgery and radiotherapy, there is still debate regarding the use of these drugs as first line therapy. In a retrospective study using three times daily subcutaneous octreotide, Newman *et al*. found no significant difference in GH and IGF-I reduction between primary and secondary treatment groups (252), suggesting that octreotide may be a reasonable primary medical option under certain circumstances, provided the tumour does not threaten vision or neurological function. In our study, the subgroup of patients on primary medical therapy achieved similar

levels of GH and IGF-I suppression compared with the group as a whole. Using GH criteria 40% of subjects achieved "remission" at latest follow-up and 60% achieved normal IGF-I concentrations. These figures are similar to those observed in the whole group (36% and 62% respectively). There was no statistically significant difference in GH levels between the primary and secondary treatment groups at baseline, after 12 months of treatment or at latest follow-up and the same was true for IGF-I. The safety profile in the primary therapy group was no worse than in the group as a whole, with only one patient developing gallstones and no change in overall glucose tolerance. Interpretation of our results must take into account the fact that most patients in the primary therapy group had microadenomas on pituitary imaging, although there was no statistically significant difference in baseline GH and IGF-I levels between the two groups.

As with previous studies, I observed a discrepancy between the proportion of patients with "safe" GH levels and those with normal IGF-I levels. There is ongoing debate about the correlation (or lack thereof) between serum GH levels and serum IGF-I levels in individual patients treated for acromegaly and a number of mechanisms have been proposed to explain these discrepancies. IGF-I is dependent on the pattern as well as the amount of GH secretion (156) and may be affected differently from GH by different treatment modalities. In addition, several non-GH dependent factors and mechanisms contribute to the determination of serum IGF-I levels (253). This is discussed in greater detail in Chapter 3.

To conclude, in this study I demonstrated that long-term use of depot long-acting somatostatin analogues for the treatment of acromegaly is effective and safe, with the outcomes observed in short term studies sustained over longer periods. There was no increase or worsening of the observed side effects and no increase in tumour size with long-term use, although the

development of impaired glucose tolerance in 4 patients at a time when GH levels were either improving or static highlights the ongoing need to monitor the long-term safety of these preparations. Our findings also support the mounting evidence that in a subgroup of patients in whom surgery is unlikely to result in a "cure", long-term treatment with depot somatostatin analogues is a safe and effective option, provided the tumour does not threaten vision or neurological function.

5.3 Factors determining the use of primary medical therapy in acromegaly

5.3.1 Introduction

The development of long-acting formulations of somatostatin analogues has led to improved patient compliance and comfort. Somatostatin analogues are therefore increasingly being used as first-line therapy for acromegaly, and in the previous two sections (Section 5.1 & Section 5.2), I demonstrated that GH and IGF-I suppression is similar in patients receiving these agents as primary therapy and in those in whom they are used as an adjunct to surgery and/or radiotherapy. Several papers in recent years have reported on the efficacy of primary pharmacotherapy with somatostatin analogues in achieving biochemical disease control (71,72,235,236,254,255). Safe GH levels are achieved in around 64% of patients, and IGF-I normalisation occurs in a similar proportion (256). However, somatostatin analogues are costly, and involve long-term monthly injections; careful patient selection is therefore required. Primary medical treatment can be offered to patients with large invasive tumours who have no evidence of central compressive effects (as the likelihood of surgical cure is low), those with high surgical or anaesthetic risk, and

those who decline surgery (257). Primary medical treatment is also appropriate in patients with no evidence of an adenoma or with empty sella turcica on MRI scanning. The characteristics of patients managed with primary medical therapy vary between different centres, and there are no clear criteria to help predict which patients are likely to respond to primary medical therapy. In this project I examined the diagnostic criteria upon which the decision to treat patients with acromegaly with primary medical therapy were based and evaluated outcome in these patients.

5.3.2 Subjects and methods

I retrospectively analysed data from a cohort of 15 patients with acromegaly (13 female) treated with depot somatostatin analogues as primary medical therapy. All subjects were treated either with Sandostatin LAR (Novartis), Lanreotide LA (Ipsen) or Lanreotide Autogel (Ipsen) and doses were titrated according to biochemical response. In total 10 subjects were treated with Octreotide LAR (9 with a dose of 20 mg every 4 weeks and 1 with 30 mg every 4 weeks), 1 with Lanreotide LA 30 mg every 2 weeks and 4 with Lanreotide Autogel (2 with a dose of 90 mg every 4 weeks and 2 with 120 mg every 4 weeks). Mean age at diagnosis was 59 years (range 43-83) and mean duration of treatment 55 months (8-99). 8 patients had microadenomas and 3 had macroadenomas. In 2 subjects, pituitary MRI scans were reported as normal, and 2 subjects had an empty sella. No patient had visual signs or symptoms.

Serum GH and IGF-I levels were measured as described in Section 5.2.3. Pituitary MRI scans were performed at baseline and at regular intervals.

5.3.3 Statistical analysis

Measurements of GH and IGF-I are expressed as the mean ± SEM and the Wilcoxon matched-pairs signed ranks test was used to assess the effect of treatment on biochemical parameters. A value of $p < 0.05$ was considered statistically significant.

5.3.4 Results

Decision to treat with primary medical therapy was due to patient preference in 3 cases (20%) and physician recommendation in 12 cases (80%). Reasons for physician recommendation included low probability of surgical cure, absence of a discrete adenoma, excellent response to subcutaneous octreotide, and the presence of significant comorbidity which would increase anaesthetic and surgical risk (Table 5.4).

Reason	n
Absence of a discrete adenoma	4
Excellent response to sc octreotide	4
Low probability of surgical cure	3
Presence of significant comorbidity	1

Table 5.4: Reasons for physician recommendation for primary medical therapy

Mean GH fell from 14.8 ± 3.6 μg/L at diagnosis to 2.1 ± 0.6 μg/L at latest follow up ($p<0.0001$). Figure 5.11 shows mean serum GH levels at baseline and at latest follow up in all subjects. 80% of patients achieved GH levels < 2.5 μg/L and 53% achieved a GH nadir of < 1 μg/L during a glucose tolerance test.

Mean IGF-I was 773 ± 60 μg/L at diagnosis, falling to 215 ± 24 μg/L at latest follow up ($p=0.0001$). Figure 5.12 shows serum IGF-I levels at baseline and at latest follow up in all subjects. 67% achieved a normal age-matched serum IGF-I.

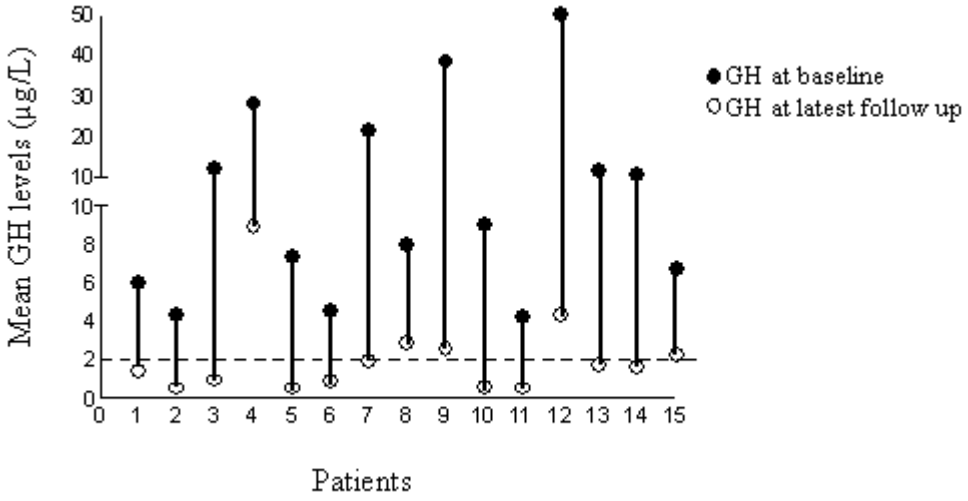
Significant tumour shrinkage (>20%) occurred in 36% (4/11) of patients with lesions on their original scans, with no cases of tumour enlargement. No patients developed deficiencies of any other pituitary hormones. New gallstone formation occurred in only one case.

5.3.5 Discussion

There is now significant evidence that primary medical therapy in acromegaly results in normalisation of GH and IGF-I together with substantial tumour shrinkage in a significant proportion of patients (14). However, there are no clear criteria for predicting which patients are likely to respond to primary medical therapy, and the reasons for employing such treatment vary in different centres.

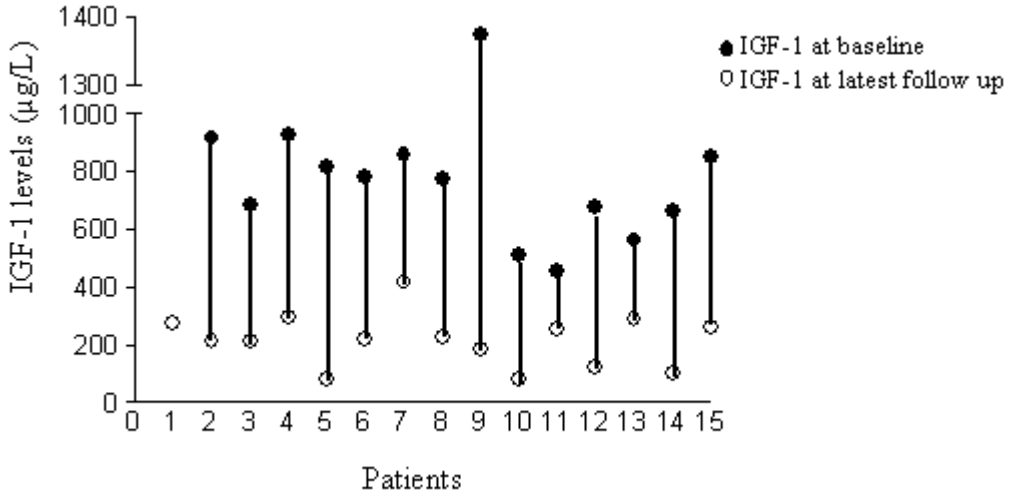
In this study of 15 *de novo* patients with acromegaly, I found that the decision to treat with primary medical therapy was predominantly physician-driven. The reasons given varied from low probability of surgical cure to absence of a discrete adenoma, an excellent response to subcutaneous octreotide, and the presence of significant comorbidity.

Figure 5.11



Serum GH levels at baseline and at latest follow up in all subjects

Figure 5.12



Serum IGF-I levels at baseline and at latest follow up in all subjects

It was clear the decision to treat with primary medical therapy was based on a careful assessment of benefit and risks in each individual patient.

I observed a significant reduction in GH and IGF-I levels in the whole group at latest follow-up, with 80% of patients achieving a "safe" GH level of < 2.5 μg/L and 67% achieving a normal age-matched serum IGF-I. Of the 3 patients who did not achieve a GH < 2.5 μg/L, 2 had GH levels > 25 μg/L at diagnosis. The findings that the pre-treatment GH levels influence efficacy of somatostatin analogues has been demonstrated in other studies; when patients were grouped according to pre-treatment GH levels, those with higher initial GH concentrations were less likely to normalise GH and IGF-I during treatment (72,228). Not all studies, however, support this notion (236), and neither did our study reported in Section 5.2.

Although reports have been limited by a lack of rigour in study design, heterogeneous imaging techniques and measurements, and a lack of controls, both lanreotide and octreotide, either in the subcutaneous or in the slow release intramuscular formulation, have already been shown to be effective in reducing tumour size, and, so far, very few cases of tumour growth during somatostatin analogue therapy have been reported. Clinical trials reporting changes in tumour size associated with somatostatin analogue treatment for acromegaly have used different criteria to define significant tumour shrinkage; in most studies, this has ranged from 20 to 45% reduction in tumour size.

A recent meta-analysis of 14 studies with 424 subjects showed that > 36% of patients receiving primary somatostatin analogue therapy for acromegaly experienced a significant reduction in tumour size (73). In our cohort, we observed significant tumour shrinkage in a similar proportion of patients (36%); amongst these were two cases in which a pituitary microadenoma disappeared completely following treatment. These findings raise the question of whether preoperative

primary somatostatin analogue treatment might improve surgical cure rates. Improvement of surgical results has been reported from some historical comparisons (258,259) and from retrospective analyses (260). Other authors, however, have seen no difference in surgical cure rates between pretreated and untreated patients (261-265). These discrepancies have led to uncertainty regarding the value of somatostatin analogue pretreatment of patients undergoing surgery.

In the only prospective, randomised study to address this issue, Carlsen *et al*. randomised 62 patients with acromegaly to either directly undergo transsphenoidal surgery (n = 30) or receive pretreatment with octreotide LAR for 6 months prior to surgery (n = 32) (266). Surgical cure rate defined by IGF-I normalisation was greater in the pretreated subjects (45%) than in those that did not receive preoperative octreotide (23%). This was most marked in subjects with macroadenomas (50% cure rate in the pretreated group *versus* 16% in those undergoing direct surgery). Although these findings suggest pretreating patients with acromegaly with a somatostatin analogue for 6 months may be beneficial, a number of issues were raised; at 34%, the overall surgical cure rate in this study was much lower than the rates generally reported in the literature, and the benefit of pretreatment disappeared when both GH and IGF-I were used to assess response. In addition, final hormonal assessment was performed only 3 months postoperatively, at which time there may still be some lingering effect from the somatostatin analogue.

Patients with acromegaly are at risk of anaesthetic morbidity, with increased rates of anaesthetic-associated haemodynamic changes and difficult intubations (267). There is some evidence that preoperative treatment with a somatostatin analogue may help reduce this risk. Colao *et al*. found that short-term (3- to 6-months) presurgical treatment with octreotide in 59 patients with

acromegaly resulted in disappearance of ECG abnormalities, improved responses to antihypertensive and antidiabetic drugs, and normalisation of blood glucose, total cholesterol and triglyceride levels (76). Pretreated patients also had shorter postoperative hospital stays.

These findings suggest that at present there is insufficient evidence to recommend routine preoperative use of somatostatin analogues to improve surgical outcome. However, in individual patients, consideration needs to be given to potential improvements in perioperative morbidity and reduction in anaesthetic risk which could be achieved with preoperative treatment.

Secretion of pituitary hormones depends on the integrity of the hypothalamus, portal vessels, pituitary stalk and hormone secreting cells of the pituitary. Partial or complete loss of normal pituitary function (hypopituitarism) has been recognised for many years as one of the major clinical manifestations not just of pituitary adenomas, but also of their treatment (surgery and radiotherapy). A number of studies have examined mortality in patients with hypopituitarism and all have confirmed increased mortality compared with age-matched controls, predominantly due to respiratory, cardiovascular, and cerebrovascular disease (134,178-180). In Chapter 3 we demonstrated a trend towards reduced survival in patients with acromegaly with the greatest number of deficient axes; it is therefore significant that in this cohort of patients treated with primary medical therapy, no patients developed deficiencies of any other pituitary hormones following treatment. Two patients required hydrocortisone replacement at diagnosis, but following treatment with a somatostatin analogue there was recovery of the hypothalamo-pituitary-adrenal axis.

The absence of hypopituitarism in this cohort may in part be due to the fact that most patients had microadenomas; however, these finding were also observed in a recent prospective study

examining the impact of long-term treatment with primary medical therapy in acromegaly on disease activity and tumour size (236).

Our findings confirm the efficacy and safety of depot somatostatin analogues when used as primary medical therapy for acromegaly in certain patients, and suggest GH at diagnosis, but not tumour size, may predict outcome. There are currently no clear evidence-based guidelines to determine which patients should be managed with primary medical therapy, and the decision to treat with primary medical therapy must be based on a careful assessment of benefit and risks in each individual patient. Further studies are required to identify patients most likely to respond to primary medical therapy and to formalise the process of decision-making.

5.4 Conclusion

Given the limitations of the other therapeutic modalities, medical therapy is increasingly being used in the management of acromegaly, both as primary therapy and as an adjunct to surgery and/or radiotherapy. In this chapter I have demonstrated the efficacy and safety of depot somatostatin analogues, whether used as primary or adjuvant therapy. Efficacy was maintained with long-term use and no worsening of the side-effect profile was seen. Given this proven safety and efficacy record, depot somatostatin analogues are the most widely recommended form of medical therapy for acromegaly.

However, not all patients will achieve safe GH and IGF-I levels despite treatment with surgery, radiotherapy and somatostatin analogues. The GH receptor antagonist pegvisomant has been licensed in Europe since 2003 for the treatment of patients refractory to or intolerant of somatostatin analogues. The initial placebo-controlled and open-label extension trials of

pegvisomant demonstrated normalisation of IGF-I in 90% and 97% of patients respectively (79,80). Early concerns surrounding tumour growth (analogous to the growth of ACTH-secreting pituitary adenomas in Nelson's syndrome), deranged liver function and the clinical impact of antibody formation are being addressed as experience with the use of this drug grows (82,268). Due to the high cost of pegvisomant and concerns about tumour growth, combination therapy with a somatostatin analogue has been proposed in some guidelines (165); it was hoped that lower doses of pegvisomant would be required to normalise IGF-I, at a lower cost, and the somatostatin analogue would continue to exert anti-tumoural effects and prevent tumour growth. When pegvisomant was added to the treatment regimen of 32 subjects who had not achieved IGF-I normalisation with high-dose somatostatin analogue monotherapy, IGF-I normalised in all subjects, with pegvisomant only needing to be administered once or twice weekly (269). However, there has only been one controlled study comparing pegvisomant monotherapy with combination therapy with a somatostatin analogue (270); This was a 40-week, open-label, parallel group, multicentre, randomised study which enrolled 84 patients with acromegaly who had previously undergone surgical treatment and/or radiotherapy, and had been receiving long-acting octreotide for at least 6 months. Of these, 56 patients with inadequately controlled disease were randomised to pegvisomant monotherapy or combination therapy with pegvisomant and long-acting octreotide, and 28 patients who were adequately controlled on long-acting octreotide were assigned to a control group. Both monotherapy and combination therapy were well tolerated and there were no differences in the number of adverse events. Normalisation of IGF-I was similar with both regimens (56% in the monotherapy group and 62% in the combination therapy group). The median pegvisomant dose in the monotherapy arm (20 mg/day) was greater than that in the combination treatment arm (15 mg/day), suggesting that, on average, combination therapy

offered no cost benefit over pegvisomant monotherapy. The authors therefore propose the decision should be based on an individual patient's characteristics; significant tumour shrinkage with a somatostatin analogue argues for combination therapy, while pegvisomant monotherapy is appropriate if glucose tolerance deteriorates with somatostatin analogue treatment.

6. PITUITARY TUMOUR PATHOGENESIS; ABNORMAL EXPRESSION OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 IN HUMAN PITUITARY ADENOMAS

6.1 Introduction

Numerous factors and alterations have been shown to influence pituitary tumourigenesis and cytodifferentiation, but the pathogenic mechanisms leading to formation of pituitary tumours remain poorly understood. Most human pituitary adenomas are thought to arise from a genetic event that alters cell proliferation or survival, such as activating mutations of G protein α-stimulating activity polypeptide (GSP), inactivation of a tumour suppressor gene such as MEN1, or overexpression of the pituitary tumour-transforming gene PTTG (9,271). These mutations allow cells to become more responsive to hormones or growth factors, promoting clonal expansion.

Glucocorticoids play important roles in normal physiology by modulating metabolic and immune responses. At a cellular level their actions are, at least in part, mediated via inhibition of cell proliferation and induction of differentiation. These responses have been demonstrated in a variety of tissues and are associated with the ability of glucocorticoids to arrest cells in G1-phase of the cell cycle (272,273). Although it is now clear that glucocorticoids regulate the transcription of a diverse array of target genes, the precise mechanisms by which glucocorticoids receptor (GR)-mediated changes in cell proliferation and differentiation occur are still far from clear. Although circulating levels of glucocorticoids are sensitively maintained by the hypothalamo-pituitary-adrenal axis during normal physiology, ‘pre-receptor’ or ‘intracrine’ regulatory

mechanisms have been described for several steroid hormones that involve target tissue activation or inactivation of the circulating hormone (274). For glucocorticoids, tissue-specific metabolism is catalysed by two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) (275,276) that interconvert biologically active cortisol and inactive cortisone. 11β-HSD1 is a low-affinity NADP+/NADPH-dependent enzyme that acts predominantly as an oxo-reductase in glucocorticoid target tissues such as the liver, gonads and adipose tissue. It converts cortisone to cortisol, thus regulating the level of active glucocorticoid available to the glucocorticoid receptor. In contrast, 11β-HSD2 is a high affinity NAD+-dependent dehydrogenase found predominantly in mineralocorticoid responsive tissues such as kidney, salivary gland and colon, which converts cortisol to cortisone, thereby protecting the mineralocorticoid receptor (MR) from cortisol excess (275,276). Thus the two 11β-HSD isozymes act as critical regulators of glucocorticoid action at a tissue level and thereby may have key roles in determining effects on cell proliferation and differentiation.

In previous studies, the expression of 11β-HSD isozymes has been documented in both rodent and human pituitaries (277-279). 11β-HSD1 was co-localised to growth hormone- (GH) and prolactin- (PRL) secreting cell types in the normal human pituitary and immunohistochemistry revealed staining for 11β-HSD1 in pituitary tumours derived from cells of these origins. 11β-HSD2 protein, however, was undetectable in normal pituitaries but was readily detectable in pituitary tumours, including corticotrophin- (ACTH) secreting tumours (279) (Figure 6.1).

The function of 11β-HSD in the pituitary has yet to be fully clarified and may include an additional level of endocrine regulation within the established hypothalamic-pituitary-adrenal axis. Given the recent observations of increased 11β-HSD2 protein in a small number of pituitary tumours (279) and the potentially central role of glucocorticoid signalling in determining cell

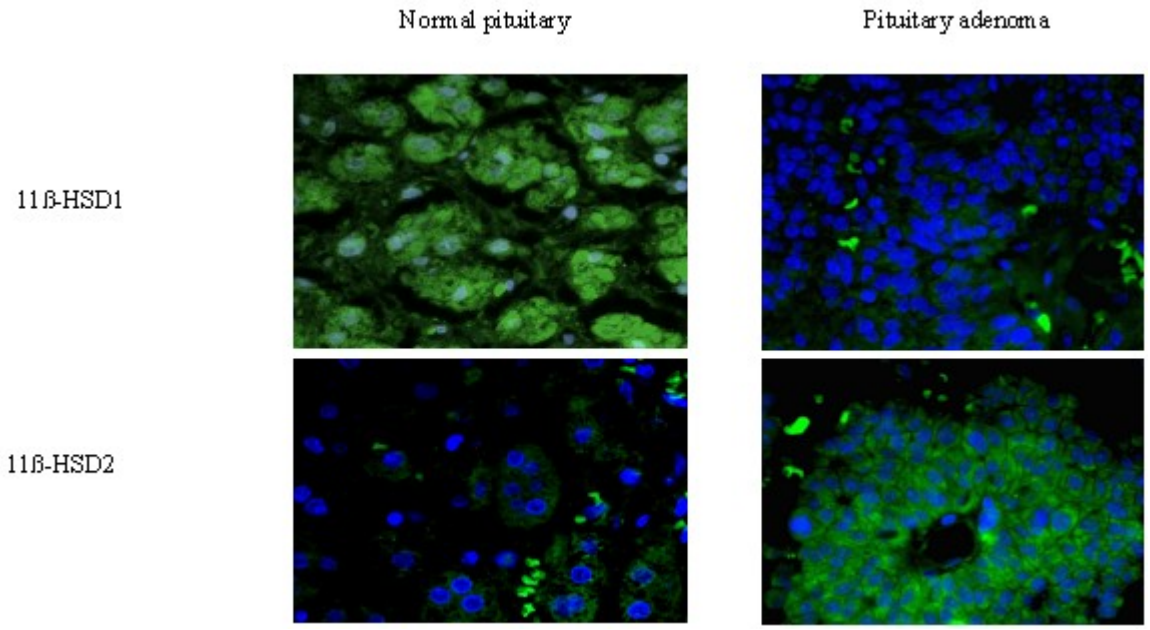
proliferation and differentiation, we hypothesised that 11β-HSD may play a role in pituitary tumourigenesis. I therefore carried out studies to characterise mRNA levels for 11β-HSD1 and 2 in a large cohort of normal and neoplastic pituitary tissue using the highly accurate and reproducible technique of quantitative real time RT-PCR.

6.2 Materials and methods

6.2.1 Patients and pituitary tissues

Pituitary adenoma tissue was obtained at the time of transsphenoidal or transcranial surgery (with the approval of the local ethics committee) and was immediately snap frozen. Clinical details of the patients who consented for study of pituitary tissues are summarised in Table 6.1. Tumours were classified on clinical and endocrinological criteria and immunohistochemical staining for anterior pituitary hormones was performed. In total there were 105 pituitary adenomas (51 (49%) from male patients; age of patients overall was 60 ± 1 years, mean ± SE); 76 non-functioning adenomas (NFA), 15 GH-secreting, 6 ACTH-secreting, 5 PRL-secreting and 3 TSH-secreting adenomas. 10 normal pituitaries were also examined; these had been obtained from post-mortems carried out within 24 hours of death on patients with no evidence of underlying endocrine disease. All pituitary samples were stored at -80°C. Previous studies have shown material collected in this way to be suitable for mRNA and protein expression studies (280).

Figure 6.1



Immunohistochemistry for 11β-HSD1 and 11β-HSD2 (both in green) in normal pituitary (left panel) and in a corticotroph adenoma (right panel). There is positive staining for 11β-HSD1 and negative staining for 11β-HSD2 in normal pituitary, and vice versa in the corticotroph adenoma. Nuclei are counterstained with blue. Magnification, x100 [adapted from (279)]

	Age ± SE	Females (%)	Macroadenomas (%)
All tumours (n = 105)	59.0 ± 1.4	53 (51)	94 (90)
Non-functioning (n = 76)	62.0 ± 1.6	38 (50)	76 (100)
GH-secreting (n = 15)	55.4 ± 3.1	9 (60)	10 (67)
PRL-secreting (n = 5)	37.7 ± 0.3	2 (40)	3 (60)
ACTH-secreting (n = 6)	41.4 ± 6.3	3 (50)	1 (17)
TSH-secreting (n = 3)	56.0 ± 6.1	1 (33)	3 (100)

Table 6.1: Patient details of full cohort showing tumour subtype, age (± SE) at diagnosis, number (%) of females, and number (%) of macroadenomas (>1 cm diameter).

6.2.2 RNA extraction, reverse transcription and quantitative PCR

Total RNA was extracted from approximately 100 mg of pituitary tissue utilising the Sigma Trisol kit - a single step acid guanidinium phenol-chloroform extraction procedure (281)- following manufacturer's guidelines as previously described (282). RNA was reverse transcribed using avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Madison, WI) in a total reaction volume of 20 µl, with 1 µg of pituitary total RNA, 30 pmol of oligo (dT)15, 4 µl of 5x AMV reverse transcriptase buffer (Promega), 2 µl of deoxynucleotide triphosphate (dNTP) mix (200 µM each) (Boehringer Mannheim, Germany), 20 units of ribonuclease inhibitor (RNasin®, Promega) and 15 units of AMV reverse transcriptase (Promega).

Gene expression of the 11β-HSD isozymes was determined using the ABI PRISM 7700 Sequence Detection System, which employed TaqManTM chemistry for highly accurate quantification of mRNA levels (283). RT-PCR was carried out in 25 µl volumes, in a reaction buffer containing 2x TaqMan Universal PCR Master Mix (consisting of 3mM Mn(OAc)2, 200 µM dNTPs, 1,25 units AmpliTaq Gold polymerase and 1.25 units AmpErase UNG), 125 nmol TaqMan probe and 900 nmol primers. Probe and primer sequences are listed in Table 6.2.

	Forward Primer	Reverse Primer
11β-HSD1	AGGAAAGCTCATGGGAGGACTAG	ATGGTGAATATCATCATGAAAAAGATTC
	Probe CATGCTCATTCTCAACCACATCACCAACA	
11β-HSD2	GGGCCTATGGAACCTCCAA	GACCCACGTTTCTCACTGACTCT
	Probe CCGTGGCGCTACTCATGGACACA	

Table 6.2: Oligonucleotide sequences of PCR primers and TaqManTM probes used. All TaqMan primers run at 59 °C and yield amplicons of 70 – 150 bp

All reactions were multiplexed with 18S ribosomal RNA, provided as a pre-optimised control probe (PE Biosystems, Warrington, UK). This compensates for differences in RT efficiency by enabling data to be expressed in relation to an internal reference. As per the manufacturer's guidelines, data were expressed as Ct values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine ΔCt values (ΔCt = Ct of the target gene

(e.g. 11β-HSD1) minus Ct of the housekeeping gene). Lower ΔCt values indicate higher mRNA expression. To exclude potential bias due to averaging data that had been transformed through the equation $2^{-\Delta\Delta Ct}$ to calculate fold changes in gene expression, all statistics were performed at the ΔCt stage. Measurements were carried out on a minimum of two occasions for each sample. Reactions were as follows: 50ºC for 2 minutes, 95ºC for 10 minutes; then 44 cycles of 95ºC for 15 seconds and 60ºC for 1 minute.

6.2.3 Association between gene expression and clinical findings

Detailed analyses of pre-operative MRI scans were made in 87 of the 105 patients (82%) in whom informative data had been obtained from molecular studies on pituitary adenoma tissue. The baseline clinical characteristics of patients in this subgroup were not significantly different from the total cohort of 105. There were 66 patients (age 61.1 ± 1.6 years, mean ± SE, 33 female) with NFAs, 8 (12%) of whom had had repeat surgery performed for re-growth of tumour. 9 (14%) patients had deficiency of at least 1 pituitary hormone at presentation. 14 patients (age 56.9 ± 3.4 years, mean ± SEM, 9 female) had GH-secreting tumours and 1 (7%) received surgery for recurrent disease. There were 4 patients (age 37.5 ± 0.5, mean ± SEM, 2 female) with prolactinomas, and 2 (age 47.5 ± 12.5, mean ± SEM, 1 female) with ACTH-secreting adenomas. 1 patient (age 55, male) had a TSH-secreting tumour (see Table 6.3 for summary of clinical data).

Tumour (n)	Age	Female %	Recurrent %	Hypopituitary %
All (87)	59.4 ± 1.4	52	10	16
NFA (66)	61.1 ± 1.6	50	12	14
GH (14)	56.9 ± 3.4	64	7	36
PRL (4)	37.5 ± 0.5	50	0	0
ACTH (2)	47.5 ± 2.5	50	0	0
TSH (1)	61.0 ± 6.0	50	0	0

Table 6.3: Patient details and evidence of tumour re-growth (recurrent) and hypopituitarism pre-operatively (hypopituitary) in patients in whom radiological assessment of tumour size and invasion was made. Mean age in years ± SE. All – all pituitary tumour data combined, NFA – non-functioning adenoma, GH – GH-secreting tumour, PRL – PRL-secreting tumour, ACTH – ACTH-secreting tumour, TSH – TSH-secreting tumour.

Pituitary tumour volume was calculated in coronal and sagittal planes from MRI scans performed at initial clinical presentation. The previously validated SIPPAP classification (284) was used to score pituitary tumours for evidence of extension and invasion into surrounding structures. Patient's demographic details and pituitary function pre- and postoperatively were recorded, as was a history of previous surgery or radiotherapy for a pituitary tumour. Data were examined to determine any associations between clinical tumour behaviour and pituitary gene expression.

6.3 Statistical analysis

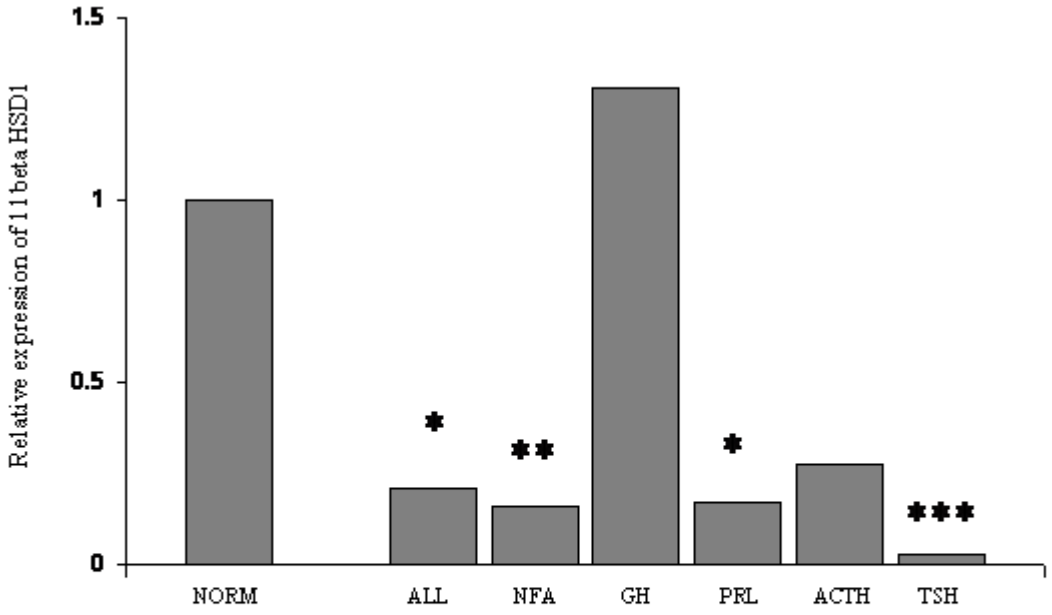
Data were analysed using Minitab version 13 software. The Student t-test was used for between group comparisons and this was performed at the ΔCt stage to exclude potential bias caused by averaging data that had been transformed through the equation $2^{-\Delta\Delta Ct}$. Significance was taken as $P < 0.05$.

6.4 Results

6.4.1 11β-HSD1 and 2 mRNA expression in pituitary adenomas

Both 11β-HSD1 and 2 mRNAs were expressed in normal pituitary tissue. Overall, pituitary adenomas expressed significantly lower levels of 11β-HSD1 mRNA compared with normal pituitary tissue (0.2-fold, $p < 0.05$, Figure 6.2), and this was true for ACTH-, PRL-, TSH-secreting, and non-functioning adenomas, but not for GH-secreting adenomas (1.3-fold excess, p = N/S), perhaps reflecting the localisation of 11β-HSD1 to predominantly somatotropes in the normal pituitary (279). In contrast, expression of 11β-HSD2 mRNA was 9.8-fold ($p < 0.001$) greater in the total group of pituitary tumours than in normal pituitaries (Figure 6.3). ACTH-secreting adenomas had the lowest expression of 11β-HSD2 mRNA within the group of tumours, although greater than in normal pituitaries at 2.5-fold (p = N/S). GH-secreting tumours expressed 8.2-fold excess ($p < 0.01$), PRL-secreting tumours 8.3-fold excess ($p = 0.05$), and TSH-secreting tumours 3.0-fold excess (p = N/S). The greatest expression of 11β-HSD2 mRNA was observed in NFAs with 12.1-fold excess ($p < 0.001$).

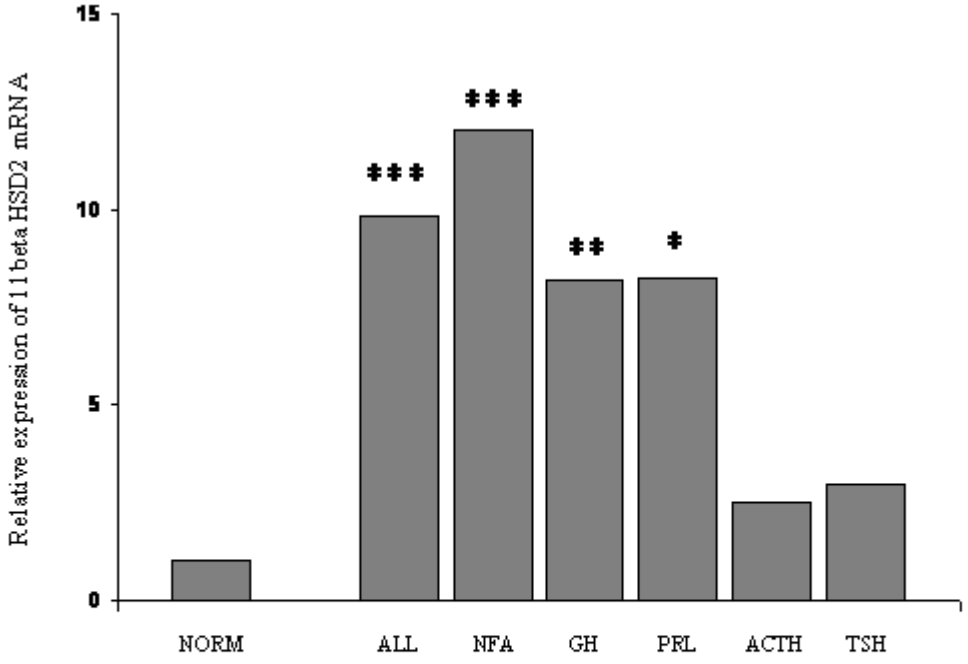
Figure 6.2



	NORM	ALL	NFA	GH	PRL	ACTH	TSH
Number	10	102	74	15	5	5	3
Mean dCT ± SEM	12.56 ± 0.48	14.84 ± 0.30	15.25 ± 0.30	12.17 ± 0.80	15.12 ± 1.23	14.38 ± 1.32	18.37 ± 2.11

*Relative levels of expression of 11β-HSD1 mRNA in normal pituitaries and pituitary tumours calculated from the mean ΔCT values shown in table below (see Materials and Methods Section). Expression of 11β-HSD1 mRNA in normal pituitaries (Norm) was normalised to 1 and compared to all pituitary tumours collectively (All) - 0.2-fold (*p<0.05), non-functioning adenomas (NFAs) - 0.2-fold (**p<0.01), GH-secreting (GH) - 1.3-fold (p=N/S), PRL-secreting (PRL) - 0.2-fold (*p<0.05), ACTH-secreting (ACTH) - 0.3-fold (p=N/S), and TSH-secreting (TSH) pituitary adenomas 0.02-fold (***p=0.001).*

Figure 6.3



	NORM	ALL	NFA	GH	PRL	ACTH	TSH
Number	10	105	76	15	5	6	3
Mean dCT ± SEM	15.93 ± 0.85	12.64 ± 0.28	12.35 ± 0.35	12.90 ± 0.60	12.90 ± 1.11	14.61 ± 1.12	14.37 ± 1.03

*Relative levels of expression of 11β-HSD2 mRNA in normal pituitaries and pituitary tumours calculated from the mean ΔCT values shown in table below (see Materials and Methods Section). Expression of 11β-HSD2 mRNA in normal pituitaries (Norm) was normalised to 1 and compared to all pituitary tumours collectively (All) - 9.8-fold (***p<0.001), non-functioning adenomas (NFAs) - 12.1-fold (***p<0.001), GH-secreting (GH) - 8.2-fold (**p<0.01), PRL-secreting (PRL) - 8.3-fold (*p=0.05), ACTH-secreting (ACTH) - 2.5-fold (p=N/S), and TSH-secreting (TSH) pituitary adenomas 3.0-fold (p=N/S).*

6.4.2 Associations between 11β-HSD mRNA expression and clinical parameters

Data were analysed for all pituitary tumours and for pituitary tumour subtypes to determine any associations between clinical parameters and measures of 11β-HSD1 or 2 mRNA expression. Pituitary tumours were classified according to the SIPPAP criteria (see Section 6.2.3), which is a measure of tumour size, extension, and invasion. There were no significant associations between 11β-HSD isozyme mRNA expression (or ratios of 11β-HSD isozymes) and clinical (age, sex, degree of hypopituitarism pre-operatively, and presence of recurrent tumour growth requiring further treatment) or radiological parameters of tumour invasion. In addition, no difference in 11β-HSD isozyme mRNA expression was found between micro- and macroadenomas (data not shown).

6.4.3 Preliminary enzyme activity and cell proliferation studies

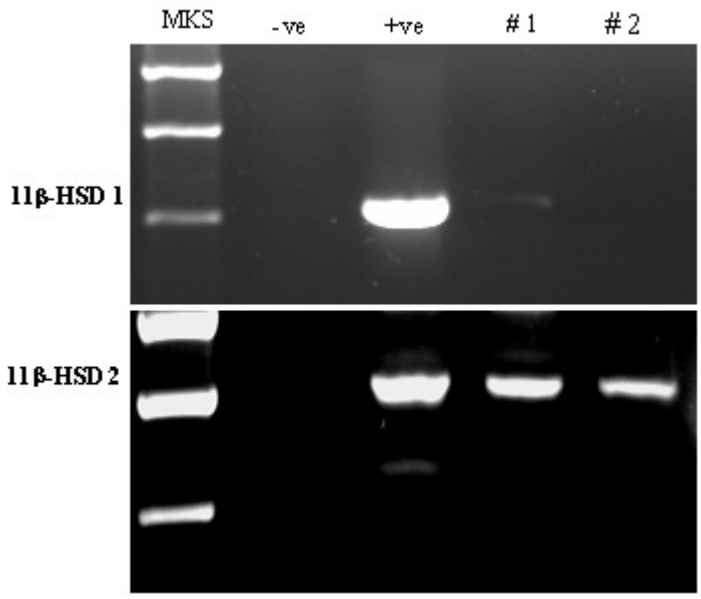
Following on from this project, preliminary enzyme activity and cell proliferation studies were carried out by another member of our group (Dr Elizabeth H Rabbitt). 6 pituitary adenomas (4 non-functioning, 2 GH-secreting) were maintained in primary culture. RNA extraction and enzyme activity studies were performed on day three. RT-PCR analyses confirmed the presence of mRNA for 11β-HSD2 but absent 11β-HSD1 in the primary tumour cultures (Figure 6.4). Likewise, 11β-HSD2 activity was readily detectable in all adenomas examined (Figure 6.5), whereas there was no detectable 11β-HSD1 activity in any of the pituitary adenomas analysed. In 4 tumours (all non-functioning) nuclear incorporation of 3H-thymidine was used to assess proliferation in pituitary tumour cells in the presence or absence of the 11β-HSD inhibitor

glycyrrhetinic acid (GE) (Figure 6.6). Using this methodology, we could measure basal pituitary cell proliferation; following treatment with GE there was a significant decrease in cell proliferation (65.5 ±15% inhibition, $p < 0.001$).

6.5 Discussion

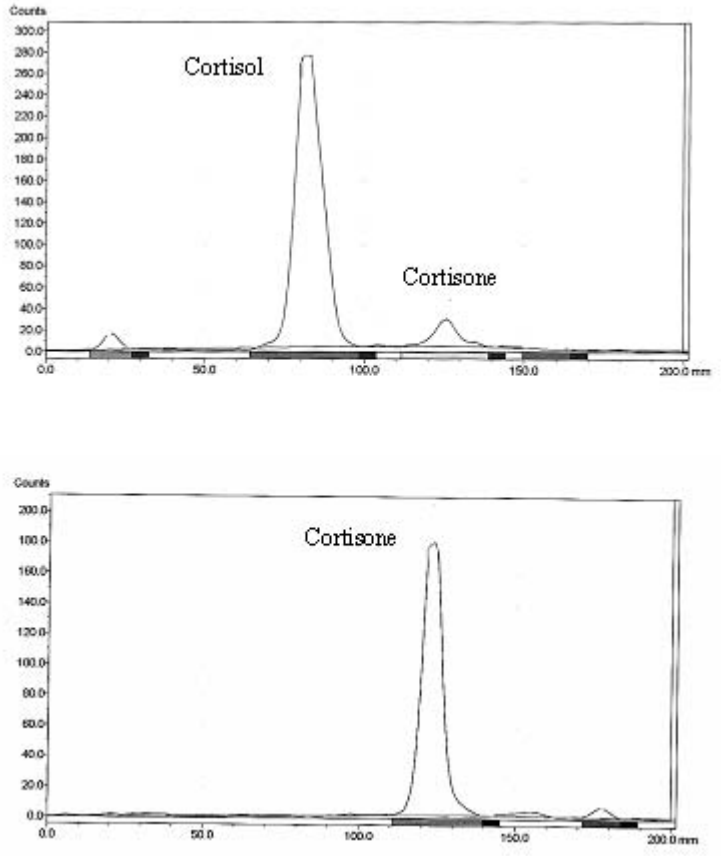
We observed a marked switch in 11β-HSD isozyme mRNA expression between normal and tumourous pituitary tissue. 11β-HSD1 expression was significantly reduced (0.2-fold compared with normals) while expression of 11β-HSD2 was significantly enhanced (9.8-fold compared with normals) in pituitary tumours. These data support the findings from a recent study of a small number of pituitary tumours in which significant 11β-HSD2 immunoreactivity in pituitary tumours but absent expression of 11β-HSD2 protein in normal pituitaries was shown (279). Initially, we postulated that a switch in isozyme expression in ACTH-secreting adenomas might account for the altered glucocorticoid feedback characterising Cushing's disease, but an even greater induction of 11β-HSD2 expression was observed in other pituitary tumours. It is unlikely, therefore, that tumour-associated changes in 11β-HSD expression play a role in glucocorticoid feedback within the pituitary. Instead we propose that a switch in 11β-HSD isozyme expression in the pituitary may confer a growth advantage and thus contribute to the process of pituitary tumourigenesis. Reduced 11β-HSD1 in pituitary tumours is likely to result in attenuated local conversion of cortisone to cortisol. Moreover, significantly increased 11β-HSD2 expression in pituitary tumours will further reduce the availability of biologically active cortisol by enhancing its inactivation to cortisone.

Figure 6.4



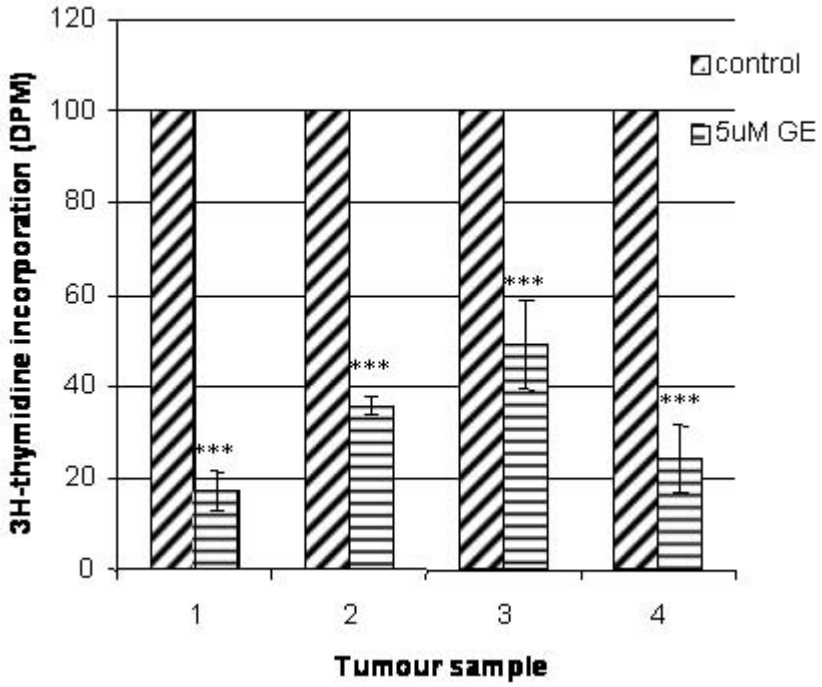
RT–PCR analysis of 11β-HSD mRNA expression in pituitary tumour cells. Lanes as follows: DNA size markers (MKS); placenta as negative control (-ve); liver as positive control (+ve); pituitary sample no. 1 (#1); pituitary sample no. 2 (#2). Results depict the presence of 11β-HSD2 but not 11-HSD1 mRNA.

Figure 6.5



Typical thin layer chromatography traces for 11β-HSD1 and 11β-HSD2 activities in primary cultures of pituitary tumour cells. Only cortisol to cortisone conversion was found indicative of 11β-HSD2 expression.

Figure 6.6



*Inhibition of pituitary tumour cell proliferation by the 11β-HSD2 inhibitor glycyrrhetinic acid (GE) (analysis of ^{3}H-thymidine nuclear incorporation). *** = significantly different from untreated cells, <0.001.*

Thus the net effect of the observed switch in 11β-HSD isozyme expression in pituitary tumours is to reduce intrapituitary glucocorticoid bioavailability. Our preliminary data showing GE-induced inhibition of cell proliferation would support these proposals.

Glucocorticoids are known to inhibit cell proliferation and induce differentiation. These responses, which have been demonstrated in a variety of tissues, are associated with glucocorticoid-mediated cell cycle arrest in the G1-phase (273). A reduction in glucocorticoid concentrations locally within the pituitary would thus diminish the anti-proliferative effects and promote pituitary cell over-proliferation and tumourigenesis. To support this concept, several recent studies have suggested a role for 11β-HSD isozymes in regulating cellular proliferation and malignancy. Elevated 11β-HSD2 expression has been described in leukaemia (285) and breast cancer cell lines (286). In the latter, the high oxidative activity of 11β-HSD2 inactivated glucocorticoids and thereby weakened their anti-proliferative action. However, addition of 18β-glycyrrhetinic acid, an inhibitor of 11β-HSD2, reduced glucocorticoid inactivation and resulted in restoration of the anti-proliferative effect, causing a 47% decrease in proliferation rates. Studies in primary bone cell cultures have revealed exclusive expression of 11β-HSD1, although in osteosarcoma cell lines, high levels of 11β-HSD2 expression were apparent (287,288). In other studies, members of our group used stable transfectants of ROS 17/2.8 osteosarcoma cells that express either 11β-HSD1 or 2 to determine effects on cell proliferation (289). A significant reduction in proliferation in 11β-HSD1 expressing cells and a significant increase in proliferation in 11β-HSD2 expressing cells was observed. The latter could be abrogated by addition of the 11β-HSD inhibitor 18β-glycyrrhetinic acid, demonstrating that the pro-proliferative effects of 11β-HSD2 were due to increased capacity for the local inactivation of cortisol. The differential responses of transfectant cells to glucocorticoids were not restricted to changes in proliferation.

11β-HSD2 transfectants showed no induction of differentiation markers (e.g. alkaline phosphatase) with cortisol, whereas 11β-HSD1 transfectants were sensitive to cortisone. 11β-HSD2 had also been shown to regulate glucocorticoid metabolism in other neoplastic tissues. In adrenal adenomas, 11β-HSD2 mRNA expression has been demonstrated to vary, depending on secretory properties of the adenoma (290). 11β-HSD2 mRNA expression was highest in non-functioning adenomas, followed by subclinical Cushing's adenomas. Lowest levels of 11β-HSD2 mRNA expression were seen in adenomas causing overt Cushing's syndrome. 11β-HSD2 mRNA levels correlated strongly with the ratio of plasma cortisone to cortisol. The authors hypothesised that 11β-HSD2 might inactivate cortisol at the site of production within some adenomas in an autocrine or paracrine manner, resulting in apparently non-functioning adrenal adenomas. Similarly, the signs and symptoms of cortisol excess in patients with adenomas causing subclinical Cushing's syndrome might be masked due to the immediate inactivation of cortisol, resulting in a prolonged and subtle clinical course until diagnosis.

COX-2 is overexpressed in colon polyps and cancer, and COX-2–derived prostaglandin E_2 promotes colorectal cancer progression (291). Both non-selective COX inhibitors (NSAIDs) and selective COX-2 inhibitors (glucocorticoids) have been shown to reduce the number and size of colonic adenomas, but are associated with significant adverse effects (291). Zhang *et al*. recently demonstrated overexpression of 11β-HSD2 mRNA and 11β-HSD2 immunoreactive protein in both human colon adenomas and in intestinal adenomas of $Apc^{+/min}$ mice displaying increased COX-2 expression and activity (292,293). Inhibition of the 11β-HSD2 enzyme using gene silencing or a pharmacological agent (glycyrrhizic acid) resulted in reduced COX-2–mediated prostaglandin E_2 production in tumours and prevented adenoma formation, tumour growth, and metastasis in mice.

The mechanisms by which glucocorticoids regulate cell proliferation are poorly understood. However, amongst the most prominent glucocorticoid target genes are the cyclin-dependent kinases (CDKs) and their corresponding CDK inhibitors (CDIs) (294). Some of these such as the Cip/Kip family of CDIs, particularly p57Kip2, are rapidly regulated by glucocorticoids and are central to the glucocorticoid-induced accumulation of cells in G1-phase of the cell cycle (273,295). Abnormal expression of CDIs has been described in human pituitary tumours (296,297) and has also been implicated in the genesis of pituitary tumours in mice (298-300). Interestingly, mice lacking both p18 and p27Kip1 rapidly develop fatal pituitary adenomas (301). Glucocorticoids may also alter cell cycling by modulating growth factor-mediated changes in tyrosine kinase signalling, either by direct effects on membrane receptor expression or by indirect regulation of protein phosphorylation (302). Thus, a switch in 11β-HSD expression may impact on several signal transduction pathways associated with cell proliferation and further studies to clarify this mechanism are required.

It is interesting to note that the only pituitary tumours expressing significant levels of 11β-HSD1 mRNA were GH-secreting tumours. This supports the findings of a previous study in which 11β-HSD1 mRNA and protein were found in somatotroph adenomas (279). Glucocorticoids play an important role in the hypothalamo-pituitary GH secretory axis; physiological amounts of these steroids are necessary for normal GH synthesis and secretion (303-305). However, chronic exposure to supraphysiological levels causes growth retardation and a decrease in GH release in response to various stimuli (306). In humans, several reports have shown that glucocorticoid excess decreases GH response to GHRH (307,308). At the same time, *in vitro* studies have demonstrated that glucocorticoids increase the synthesis and content of GH in dispersed pituitary cells, as well as sensitising somatotrophs to release GH after stimulation (309). In the pituitary,

glucocorticoids increase GH gene transcription and GH mRNA levels, and an effect on GHRH receptor mRNA has also been described (309). The presence of both 11β-HSD1 and 11β-HSD2 within the somatotrophs may be necessary to achieve and maintain adequate glucocorticoid levels for normal GH synthesis and secretion. As demonstrated in our study, this balance is altered in patients with acromegaly, with a significant increase in expression of 11β-HSD2 leading to a reduction in intra-somatotroph cortisol levels.

Expression of all subtypes of the somatostatin receptors (sst), except sst4, has been observed in GH-secreting adenomas (218). However, endogenous somatostatin suppression of GH occurs via receptor subtypes 2 and 5, and these are also the predominant types of somatostatin receptors found in GH-secreting pituitary tumours (218). Several studies have shown that glucocorticoids can influence somatostatin receptor subtype expression in a differential manner in rat and murine pituitary cells and cell lines (310-312). Xu *et al*. demonstrated that exposure of GH4C1 pituitary cells to dexamethasone for 2 hours increased sst1 mRNA levels 2.5-fold and sst2 1.5-fold compared with controls (310). Prolonged exposure (24-48 h), however, resulted in a decrease in mRNA levels of sst1 to 50% and sst2 to 30% of controls. In contrast, sst3 mRNA levels decreased initially, but increased dramatically with dexamethasone exposure > 24 hours. Using multiplex RT-PCR, Park *et al*. demonstrated increased pituitary sst2 mRNA levels following adrenalectomy, indicating that pituitary sst2 synthesis is normally under inhibitory control of endogenous glucocorticoids (311). Unlike Xu *et al*., they also found that short-term exposure of primary rat pituitary cell cultures to dexamethasone resulted in decreased sst2 mRNA levels and increased sst5 mRNA levels, suggesting dexamethasone regulation of sst2 and sst5 *in vivo* is at least in part due to direct action at the level of the pituitary. Although the findings of these 2 studies are not entirely consistent, they do show that glucocorticoids play a significant role in

regulating somatostatin receptor expression, particularly sst2 and sst5, the predominant receptor subtypes found in GH-secreting tumours. The shift in balance between 11β-HSD1 and 11β-HSD2 seen in these tumours is bound to have a significant impact on somatostatin receptor expression, raising intriguing possibilities of how it could be manipulated in patients with acromegaly for therapeutic purposes.

Our current observations and the supporting data that 11β-HSD2 may have a pro-proliferative effect in some neoplasms raises the intriguing possibility of novel therapeutic strategies to modulate tumour growth. There have been previous reports of anti-proliferative and potent anti-cancer properties of the liquorice derivatives glycyrrhizin and glycyrrhetinic acid (313,314), which are inhibitors of 11β-HSD activity. A Japanese herbal medicine “Sho-saiko-to” has also been reported to have potent anti-cancer properties (315) and one of the key ingredients of this compound is glycyrrhizin. Clearly, however, the systemic delivery of such an agent would cause unwanted effects in other 11β-HSD2 dependent tissues such as the kidney (316). An alternative and possibly more desirable approach may involve targeted gene delivery of vectors encoding 11β-HSD1 in order to reconstitute normal glucocorticoid responsiveness in the pituitary.

7. COMPLICATIONS OF PITUITARY ADENOMAS; PITUITARY APOPLEXY

7.1 Introduction

Pituitary apoplexy presents as a constellation of acute clinical features that include headache, visual deficits, ophthalmoplegia, and alteration in mental status resulting from sudden haemorrhage or infarction of a pituitary adenoma. Pituitary apoplexy as a clinical entity has been recognised for over 100 years (17) although it occurs only infrequently as a complication in patients with (usually large) pituitary adenomas (0.6 to 9.1% of cases (18-24,317)). Subclinical haemorrhage and infarction of pituitary adenomas may be detected on routine imaging, and patients may experience only mild or no symptoms to suggest an apoplectic event. The frequency of subclinical haemorrhagic infarction may be as high as 25% (18,20,22), but does not constitute a diagnosis of pituitary apoplexy.

Apoplexy may occur as the first presentation of pituitary pathology or may occur in patients with previously diagnosed functioning (22,24,27) or non-functioning (24,318) pituitary adenomas. Most cases occur spontaneously, but apoplexy has been described associated with pathological states such as hypertension (24,319), following medical interventions (e.g. dynamic pituitary function tests (320-322), cardiac surgery (323-325), pituitary radiotherapy (20,326)), or administration of medications including anticoagulants (327,328) and bromocriptine (19,21). The pathophysiology of pituitary apoplexy is poorly understood, although a rise in intra-hypophysial pressure as a result of pituitary tumour expansion is likely to alter regional blood flow within the pituitary, disrupting vascular integrity due to hypoxia (25). Taken to its extreme this may result in haemorrhagic infarction of the pituitary – pituitary apoplexy. Other proposed mechanisms

include sudden alterations in perfusion pressure to the adenoma, exposure to systemic arterial pressure due to blood supply from the inferior hypophyseal artery to the tumour, and incomplete maturation and poor fenestration of pituitary adenoma vessels, all of which could contribute to the susceptibility for spontaneous haemorrhage and thereby represent potential mechanisms for the development of apoplexy (317).

The infrequency of pituitary apoplexy renders it a difficult subject for audit; hence there are no evidence-based standards of optimum care for these patients. The key controversy in management relates to the role of acute neurosurgical intervention. Some have favoured 'routine' early neurosurgical decompression (22-24,329,330), while others advocate a more conservative approach, especially in the absence of significant or progressive neuro-ophthalmological compromise (26,27,331).

In recent years, at University Hospital Birmingham, we have adopted a conservative approach towards patients presenting with pituitary apoplexy if there is no progressive visual or neurological deterioration. To determine whether our less-interventional approach affected long-term clinical outcome in these patients, I performed a retrospective analysis to evaluate clinical presentation, management, and clinical outcomes in a cohort of patients who presented acutely with pituitary apoplexy during the period 1994-2004.

7.2 Patients and methods

I retrospectively analysed data relating to all patients presenting to our unit with clinical symptoms and signs of pituitary apoplexy from 1994 to 2004. Thirty three patients (13 female) were identified; the mean age at diagnosis was 52 (27-79) years and the mean follow up duration

was 3.7 (0.4-10.1) years. One of the patients was previously known to have a non-functioning pituitary adenoma (diagnosed 2 years previously). This patient had not required prior debulking surgery or pituitary radiotherapy. Fifteen patients (46%) underwent transsphenoidal surgery (mean age 51 (27-79) years) and 18 (54%) were managed conservatively (mean age 54 (29-79) years). Eleven patients (33%) had a previous diagnosis of hypertension. Patients were operated on by 1 of 4 neurosurgeons.

All patients underwent assessment of anterior pituitary function on admission and at subsequent follow up. Full assessment of anterior pituitary function was made by measuring FT4, TSH, LH, FSH, testosterone, PRL, cortisol (random/early morning and some had synacthen tests performed), and IGF-I. The presence of hypopituitarism was defined by proven biochemical deficiency of at least one endocrine axis. In pre-menopausal females, gonadotrophin deficiency was assumed if the serum prolactin was normal and the patient was amenorrhoeic, and in post-menopausal females if the FSH was inappropriately low.

All patients underwent CT- or MRI scanning of the pituitary and in some cases MRI scanning was performed in patients who had undergone CT scanning at other hospitals prior to being transferred. In patients who underwent transsphenoidal surgery, histopathological examination of the surgical specimen with immunohistochemical staining for pituitary hormones was performed. On admission visual fields were assessed by simple confrontation testing, but all patients proceeded to have formal visual field charting within 24-48 hours of admission, as well as review by a neuro-ophthalmologist to document any ophthalmoplegia. These formal ophthalmological assessments were repeated at regular intervals during the admission (and at subsequent follow up) to determine whether there was improvement or worsening in vision.

7.3 Results

7.3.1 Clinical features

The main presenting symptoms and signs are listed in Table 7.1. Headache was the most common presenting feature, occurring in all but one patient. Headache was frequently accompanied by nausea, vomiting and visual symptoms, each of which occurred in over 50% of patients.

Presenting symptoms and signs	%
Headaches	97
Visual deficit	82
Nausea	75
Vomiting	53
Ocular paresis	46
Meningism	25

Table 7.1: Presenting symptoms and signs (n=33)

Table 7.2 lists the visual symptoms and signs, detailing the involvement of various cranial nerves in patients with ocular paresis. The IIIrd nerve was the most commonly affected, with palsy occurring in 41% of all patients. Twenty one percent of patients had multiple cranial nerve palsies.

Visual symptoms and signs	%
Diplopia	56
Ocular paresis	46
IIIrd nerve palsy	41
IVth nerve palsy	9
VIth nerve palsy	23
Visual field deficit	36

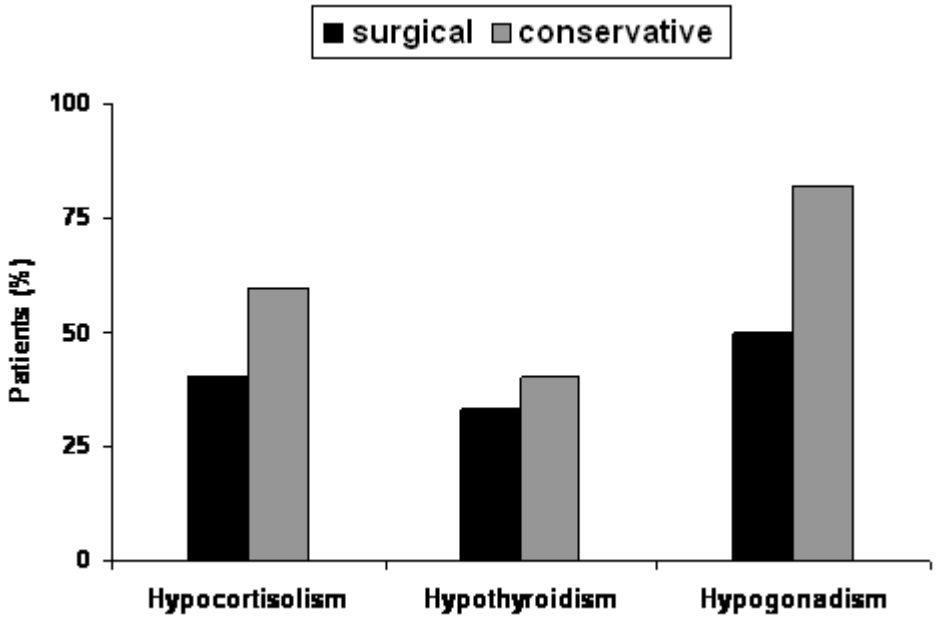
Table 7.2: Visual symptoms and signs (n=33)

The only apparent predisposing factor to pituitary apoplexy was hypertension, which was present in 33% of patients. On direct questioning, features suggestive of endocrine dysfunction were elicited in a number of patients. Eight men complained of impotence or reduced libido, and amenorrhoea was present in 4 pre-menopausal women; early menopause had occurred in a further 2. One patient complained of lethargy and cold intolerance.

7.3.2 Investigations

All patients had baseline anterior pituitary function assessment performed at presentation. Figure 7.1 shows the proportion of patients with hypogonadism, hypothyroidism, and hypocortisolism in the surgically and conservatively managed groups. Hyperprolactinaemia was present in 38% of cases (range 751- 41680 mU/L) and in 8 (24%) serum PRL was below the reference range.

Figure 7.1



Proportion of patients with hypocortisolism, hypothyroidism and hypogonadism at presentation in the surgically and conservatively managed groups (P=N/S)

CT scans were obtained in 19 patients and 14 underwent MRI scanning. On admission to our unit, MRI scans were obtained in 13 of the 19 patients who underwent CT scanning elsewhere. Haemorrhage was apparent in 22 (67%). Four pituitary adenomas and 8 cases of pituitary haemorrhage that had not been identified on CT scanning were subsequently visualised on MRI.

7.3.3 Radiological features at presentation

I was able to perform detailed assessments of initial pituitary MRI scans to classify tumour size and extension in 26 patients. The initial scans of the remaining 7 patients were not available for this detailed assessment. Using Wilson's modification of Hardy's classification (332) I encountered 10 grade 4 tumours and 6 of these were managed conservatively. Some tumours with extensive supra- and parasellar extension were also managed conservatively (Table 7.3). There was an approximately equal distribution of tumour grade and extension characteristics between the conservative and surgically managed groups. Tumour appearances on MRI were not indicative of clinical presentation in terms of visual loss and cranial nerve palsies, although such complications were less likely to be present in cases where the tumour was confined to the sella.

7.3.4 Management

Fifteen patients (46%) underwent transsphenoidal surgery and 18 (54%) were managed conservatively. Median time from presentation with apoplexy to surgery was 4 days. All but one patient was operated on within 1 to 22 days, while a single patient was referred after a prolonged period (120 days).

	Grade				Suprasellar				Parasellar		
	I	**II**	**III**	**IV**	**0**	**A**	**B**	**C**	**None**	**D**	**E**
Conservative (n=14)	1	5	2	6	3	6	2	3	1	3	10
Surgical (n=12)	0	6	2	4	1	5	5	1	3	1	8

Table 7.3: Radiological classification of tumour size and extension (n=26) based on the classification system from Hardy, modified by Wilson (see below)

Tumour classification system from Hardy, modified by Wilson (Wilson, 1979)

Grade

I sella normal or focally expanded, tumour <10 mm

II sella enlarged, tumour ≥10 mm

III localised perforation of sellar floor

IV diffuse destruction of sellar floor

V spread via cerebrospinal fluid or blood

Stage

Suprasellar extension

0 none

A occupies cistern

B recesses of third ventricle obliterated

C third ventricle grossly displaced

Parasellar extension

D intracranial (intradural)

E into or beneath cavernous sinus (extradural)

The reasons for proceeding to surgery were worsening visual deficit in 13 patients, development of hemiparesis in one patient, and decreased level of consciousness in one. Patients in whom visual deficits were stable or improving were managed conservatively. All patients received empirical glucocorticoid replacement therapy on admission.

7.3.5 Outcomes

Table 7.4 compares outcomes in the groups managed surgically and conservatively. Of the 15 surgically managed patients, 8 had cranial nerve palsies at presentation, of whom 5 (63%) made a full recovery and 7 had visual field defects that resolved in 57%. By contrast, in the group of patients managed conservatively, 7 presented with cranial nerve palsies and 6 with visual field defects, all of which resolved completely (P=N/S between groups).

Evaluation of anterior pituitary function was performed in all patients at follow up appointments. Overall, at latest follow up, 79% of patients required long-term glucocorticoid therapy and 67% required thyroid hormone replacement, compared with 50% and 37% respectively at presentation (Figures 7.1 & 7.2). Hypogonadism was present in 76% compared with 72% at presentation. There was no statistically significant difference in the rates of hypogonadism, hypocortisolism and hypothyroidism between the surgically and conservatively managed groups (Figure 7.2, Table 7.4).

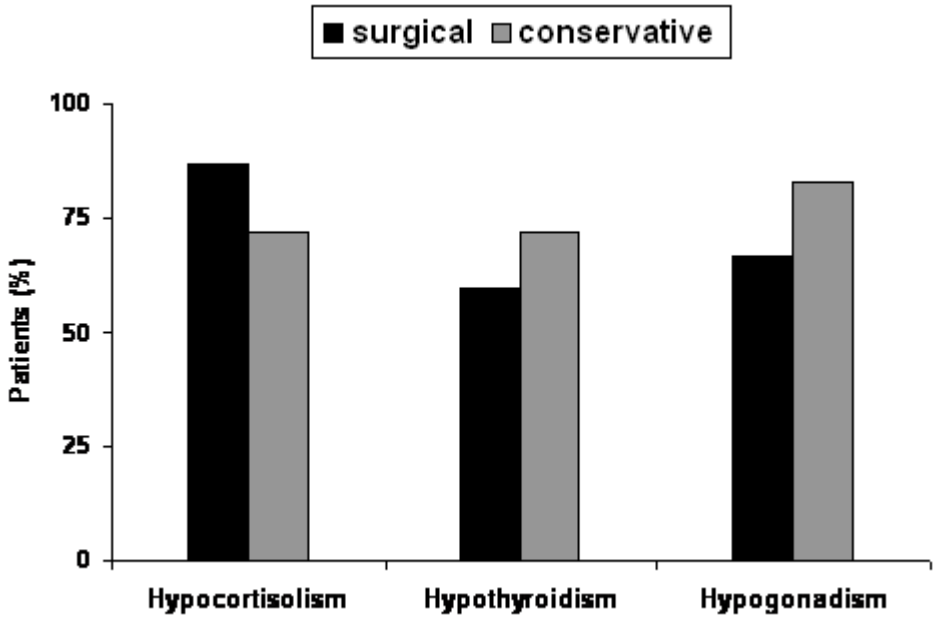
	Surgical group (n=15)	Conservative group (n=18)
Visual field recovery	57% (4/7)	100% (6/6)
Ocular palsy recovery	63% (5/8)	100% (7/7)
Hypocortisolism	87% (13/15)	72% (13/18)
Hypothyroidism	60% (9/15)	72% (13/18)
Hypogonadism	67% (10/15)	83% (15/18)

*Table 7.4***:** *Comparison of outcomes in the surgically and conservatively managed groups (P=N/S for all)*

Identification of the tumour type by histological examination and immunostaining was possible in 10 of the 15 patients who underwent surgery. Eight were non-functioning adenomas, one immunostained for growth hormone and prolactin and one displayed sparse positive staining for growth hormone. In five, the overriding appearance was extensive infarction and haemorrhage with little viable pituitary tissue identified.

Postoperatively, 3 patients (20%) developed cerebrospinal fluid leaks, 2 requiring surgical repair. One patient suffered a cardiac arrest and cerebrovascular accident during surgery and one continues to experience postoperative retro orbital pain. Six patients (60%) developed postoperative diabetes insipidus, 4 of them requiring long-term treatment with desmopressin. There were no deaths as a result of either pituitary apoplexy or surgery.

Figure 7.2



Proportion of patients with hypocortisolism, hypothyroidism and hypogonadism at follow up in the surgically and conservatively managed groups (P=N/S)

At latest follow up, one patient in the conservatively managed group had subsequently required surgery (47 months after the apoplectic event) and one of the patients managed surgically had received pituitary radiotherapy (61 months after the apoplectic event), both due to evidence of tumour re-growth on MRI.

7.4 Discussion

Pituitary apoplexy is a rare condition, meaning there is little published evidence to guide optimal management of these patients. Most published data exist in the form of case reports, and the few available cohort studies are retrospective analyses with small numbers of patients.

I have described the clinical outcome in a modern cohort of patients with pituitary apoplexy who presented to a single UK centre. A large proportion (54%) of our patients was managed without acute neurosurgical intervention and I have demonstrated that such management does not adversely influence visual or endocrine outcome. Furthermore there has been a single episode of tumour re-growth since the presenting apoplectic event in the patients managed conservatively (and one in the surgically managed group). We advocate conservative management in patients who present acutely with pituitary apoplexy in whom there is no visual deficit or evidence of spontaneously resolving visual deficit.

There continues to appear in the literature anecdotal case reports of pituitary apoplexy (325,333-335). The principal points of interest in such reports relate to atypical clinical presentations. However, the initial presentation of our cohort of patients was typical of that described by others (23,24,27,317,329-331), with a high proportion of patients having ocular paresis, a feature rarely associated with pituitary pathology other than in the setting of acute apoplexy. Whilst it is of

course important to be vigilant and aware of how pituitary apoplexy may present, it is equally important to establish how to best manage the condition once diagnosed. However, few large scale analyses of treatment and outcome exist and thus there are no evidence-based or consensus guidelines on optimum management of pituitary apoplexy in the acute setting.

There is no controversy surrounding the medical management of these acutely unwell patients, which includes careful assessment of fluid and electrolytes and correction of pituitary hormone deficiencies. The role of decompressive surgery in the context of acute pituitary apoplexy does however remain a subject of debate.

Historically, early decompression of the pituitary fossa has been advocated (23,336,337), the rationale being the possibility of better visual (23,24) and endocrine (338) outcome. One of the most extensive surveys of pituitary apoplexy from a single centre (Oxford, UK) has reported outcome in 35 patients with pituitary apoplexy (24). Eighty nine percent were treated acutely with transsphenoidal debulking surgery (*versus* 46% in our cohort). The Oxford criterion for neurosurgical intervention was evidence of neuroophthalmological complications associated with the apoplectic event. Post-operatively 86% of patients had an improvement in visual acuity. There was a significantly greater improvement in visual acuity deficits and visual field deficits (but not ocular paresis) in patients who had early (within 8 days) surgery *versus* those in whom surgery was delayed more than 8 days. In our cohort of patients 82% (100% managed conservatively and 60% managed by surgical decompression) experienced a resolution of visual deficits. However in our surgically managed group there was no difference in the rates of visual recovery whether surgery was performed before or after 8 days (data not shown). It is of course not possible to make direct comparisons between the two different cohorts from Oxford and Birmingham and neither is it appropriate to draw firm conclusions between the

conservative/surgically managed groups given the retrospective nature of this study. It is tempting to propose that those patients managed surgically may have had more significant visual compromise. Until a large scale randomised study is performed, however, the data presented herein and from Oxford provide us with the most useful information on which to formulate our clinical decision making on how best to manage pituitary apoplexy.

Some previous observational studies have also favoured a more conservative approach in the management of pituitary apoplexy when no progressive neuro-ophthalmological signs were apparent (26,27,339-341). In a small prospective study of 12 patients with pituitary apoplexy (27), Maccagnan *et al*. reported complete resolution of ophthalmoplegia in 6 of 7 patients managed conservatively, with improvement in the remaining one. In all 5 patients managed surgically, transsphenoidal surgery resulted in prompt neurological and visual improvement, but with complete recovery in only one. The rates of pituitary hormone deficiencies were similar in both groups, and tumour re-growth was observed in 2 patients, one in each group. In a larger study, Sibal *et al*. reviewed clinical presentation, management and outcomes following different therapies in 45 patients with pituitary apoplexy (331). Twenty-seven patients (60%) underwent surgical decompression, whilst 18 (40%) were managed conservatively. Patients with visual field defects were more likely than those without to be managed surgically. Complete or near-complete resolution occurred in 93-94% of the surgically treated patients with reduced visual acuity, visual field deficit and ocular palsy. All patients with reduced visual acuity, visual field deficit and ocular palsy in the conservative group had complete or near-complete recovery. Only 5 patients (19%) in the surgical group and 2 (11%) in the conservative group had normal pituitary function at follow up. One patient in the surgical group and 4 in the conservative group had a recurrence of pituitary adenoma. Similar to us, the authors concluded that patients with classical

pituitary apoplexy who are without neuro-ophthalmic signs or exhibit mild and non-progressive signs, can be managed conservatively in the acute stage.

We, like others, noted that CT scans were less reliable than MRI in making a diagnosis of pituitary apoplexy (21,23,24,331). The advent of MRI scanning has also allowed a more accurate assessment of the contents of the pituitary fossa, with identification of areas of haemorrhage and infarction that may be anticipated to resolve spontaneously without surgical intervention. Such early resolution of mass effect can sometimes be seen on repeat MRI scanning in the early days after apoplexy. We believe that the availability of MRI scanning is partly responsible for our confidence in conservative management in a significant proportion of cases of pituitary apoplexy. This study is one of few (342) that has provided detailed appearances of pituitary MRI scans at initial assessment during the apoplectic event. MRI results did not predict the likelihood of the severity of clinical presentation with ocular paresis and field defect. Patients with extensive tumours on MRI were satisfactorily managed via a conservative route. We suggest that thorough clinical assessment of the patient along with careful interpretation of MRI scan appearances allows prediction of which patients to manage conservatively/surgically.

Follow up pituitary imaging in patients managed conservatively has predominantly shown resolution of mass effect (empty or partially empty sella/normal pituitary). We witnessed tumour re-growth in two patients, one from each group. Randeva *et al*. reported tumour recurrence in 6% of patients following transsphenoidal surgery for the initial apoplectic event, and a further patient from Oxford was treated with pituitary radiotherapy following initial surgical debulking. In the study by Sibal *et al*., one patient (4%) in the surgical group and 4 (22%) in the conservative group had a recurrence of pituitary adenoma. This clearly demonstrates that all patients with pituitary apoplexy need long term endocrine and imaging follow up. There appears to be no

excess risk of tumour recurrence if patients are managed conservatively (no surgical decompression).

Some advocate that early pituitary decompression leads to improved endocrine outcome with preserved pituitary function (24,317,338). The pathology typically associated with pituitary apoplexy is haemorrhage and infarction (342,343), which is often the most striking histopathological feature. At latest follow up there was no difference between the conservatively managed and surgically managed groups with respect to the prevalence of hypopituitarism in our cohort (Table 7.4). This is similar to the findings of Sibal *et al*. (331), but other reports do suggest the impairment in pituitary hormone secretion may be reversed, at least in some patients, after surgery (338,344).

In summary, this single centre retrospective study of 33 cases of pituitary apoplexy has shown that in patients who present with no visual deficit or evidence of early improvement in visual deficit, a conservative (non-operative) approach is safe, does not result in poor visual or endocrine outcome, and is not associated with any predilection for future tumour re-growth. In conclusion we recommend careful judgement as to the necessity for pituitary surgery in patients presenting acutely with apoplexy. Close and effective communication between neurosurgeons, neuroophthalmologists, and endocrinologists is encouraged to assure optimum management of this rare but potentially life threatening condition.

8. GENERAL DISCUSSION

This work has confirmed that acromegaly is associated with increased mortality and that treatment to achieve safe biochemical targets returns mortality to that of the background population. In recent years, significant advances have been made in the management of acromegaly, resulting in a change in overall mortality rates seen in acromegaly. In a recent metaanalysis, SMR of greater than 1 was reported in all 16 studies included (90). The reported SMRs ranged from 1.16 to 3.31, with a mean weighted SMR of 1.72. A metaregression pointed towards improved survival in more recent studies, presumably due to modern treatment modalities and more strictly defined remission criteria.

This thesis and a number of other studies have demonstrated the importance of reducing GH hypersecretion to restore mortality rates in acromegaly to those of the background population. In these studies, GH levels were measured by radioimmunoassay and a "safe" threshold of 2-2.5 μg/L was identified. In recent years many institutions have switched to the use of higher sensitivity immunofluorometric, chemiluminescence and immunoradiometric assays, which have been associated with significantly lower nadir GH during OGTT in healthy controls than was previously seen with older assays (164). As GH levels measured by these highly sensitive assays are significantly lower than those measured by polyclonal radioimmunoassay, we cannot apply criteria establishing disease remission in acromegaly that were derived with older assays to GH levels measured with many assays in current use. A further factor complicating this issue is the large inter- and intra-variability of GH assays, even those in use today (162). The recent consensus statement on the standardisation of GH assays (86) will almost certainly lead to a

major revision of GH targets for monitoring treatment success and reducing mortality rates in acromegaly. Until further data are available, clinicians need to be aware of the more sensitive assays in use in their laboratories and the normal GH nadir values achieved during OGTT using these assays.

Although a number of studies in patients with pituitary tumours have suggested that treatment with radiotherapy may be associated with an increase in mortality (166,167), patients with acromegaly have been universally excluded from these studies. For the first time, I have demonstrated that patients with acromegaly are also subject to reduced life expectancy following pituitary radiotherapy. These findings have subsequently been confirmed in the Finnish Nationwide Survey of Mortality in Acromegaly, where treatment with radiotherapy was also associated with increased mortality (89).The strength of the association between increased mortality and radiotherapy is further enhanced by the fact that in both studies, the predominant cause of death in this group of patients was cerebrovascular disease. Debate surrounds the exact cause of the increased cerebrovascular mortality seen in patients treated with radiotherapy, but it is thought that radiation may cause a variety of vascular injuries and haemodynamic changes to the cerebral vasculature (173). Hypopituitarism, which is a significant problem, with around half of all patients treated with radiotherapy developing new anterior hormone deficiencies by 10 years (345), has also been implicated. The findings of this study have resulted in a change in the algorithm for the treatment of acromegaly, with radiotherapy now being reserved for patients in whom satisfactory control of tumour growth, GH and IGF-I has not been achieved by surgery and/or medical therapy.

A further part of this work has focussed on the role of somatostatin analogues in the management of acromegaly. Traditionally, transsphenoidal surgery and/or radiotherapy have been considered first line treatment for acromegaly, but with growing concerns about the link between pituitary radiotherapy and cerebrovascular mortality, the use of medical therapy, predominantly in the form of somatostatin analogues, is on the rise. I have been able to demonstrate that these agents are efficacious and safe, whether used as primary therapy or as an adjunct to other forms of therapy. I also demonstrated that the effects of treatment were maintained with long-term therapy, and were not influenced by prior surgery and or radiotherapy. Questions remain, however, about the impact of pre-surgical use of somatostatin analogues on surgical outcome, the effect of partial surgical reduction of tumour mass on subsequent responsiveness to medical treatment and the role of combination therapy with a somatostatin analogue and GH receptor antagonist.

Other medical therapies for the management of acromegaly are on the horizon. SOM230 is a new somatostatin receptor agonist which has been shown to bind all of the somatostatin receptors except sst4, and binds sst5 with an affinity 40 times greater than octreotide (346). Phase 2 studies are currently in progress, but results from a preliminary study suggest that SOM230 may offer a therapeutic benefit in a select minority of patients who would need to be identified via drug challenge or analysis of surgical specimens to define predominant sst subtypes (347). A further potential significant step in the medical treatment of acromegaly is reflected in the development of chimeric compounds that are capable of activating both dopamine and somatostatin receptors. The implication is that a chimeric molecule that retains structural components of both dopamine and somatostatin may be capable of simultaneous dual receptor activation, potentially inducing a synergistic cellular and therapeutic response (348). Clinical studies are currently underway.

Elucidating the factors involved in the initiation of pituitary tumourigenesis remains challenging. Recently, several mouse models of cell cycle regulation have revealed that pituitary tumours may show profound cell cycle dysregulation, with a number of genes implicated including p27, p16, p18 and PTTG (349). In 40% of human GH-secreting pituitary adenomas, an activating mutation of the α-subunit gene (gsp) leads to persistently activated stimulatory G-protein and high intracellular levels of cyclic AMP. This defect results in autonomous GH secretion (350). The gsp mutation is relatively specific for somatotroph tumourigenesis. As part of this project, I have demonstrated a marked switch in 11β-HSD isozyme mRNA expression between normal and neoplastic pituitary tissue. 11β-HSDl expression was significantly reduced while 11β-HSD2 expression was increased approximately 10-fold in pituitary tumours compared with normal pituitary tissue. The net effect of this switch is a reduction in active glucocorticoid concentrations locally within the pituitary, which would diminish the antiproliferative effects and promote pituitary cell over-proliferation and tumourigenesis. Subsequent studies carried out by our group confirmed these findings, with enzyme conversion data also showing that 11β-HSD activity in pituitary adenomas was exclusively due to the type 2 isozyme. In these studies, inhibition of 11β-HSD2 by glycyrrhetinic acid inhibited pituitary cell proliferation and also potentiated the antiproliferative effect of exogenously added cortisol.

Our data have highlighted the role of 11β-HSD2 as a pre-receptor regulator of pituitary cell growth, uncovering a novel tumour marker that may help to elucidate fundamental aspects of the pathophysiology of pituitary neoplasms. This raises the possibility of novel therapeutic strategies to modulate tumour growth.

In the final part of this work I investigated complications of pituitary adenomas in the form of pituitary apoplexy. Being a rare condition, there are no evidence-based standards of optimum care. Whilst most centres favour a predominantly surgical approach, in recent years we have adopted a relatively conservative approach towards patients presenting with pituitary apoplexy. In this study, I was able to demonstrate that although 54% of our patients were managed without acute neurosurgical intervention, such management did not adversely influence visual outcome, endocrine outcome or rate of tumour re-growth. We conclude that in patients who present with no visual deficit or evidence of early improvement in visual deficit, a conservative (non-operative) approach is safe. However, larger prospective trials will be necessary in the future to validate these findings and establish evidence-based guidelines for the management of pituitary apoplexy.

Appendix 1

Publications arising from this Thesis

1. **Ayuk J, Stewart SE, Stewart PM, Sheppard MC** 2002 Long-term safety and efficacy of depot long-acting somatostatin analogs for the treatment of acromegaly. *J Clin Endocrinol Metab 87:4142-4146*

2**. EH Rabbitt, J Ayuk, K Boelaert, G Westin, MC Sheppard, M Hewison, PM Stewart, NJL Gittoes** 2003 Abnormal expression of 11Beta hydroxysteroid dehydrogenase type 2 in human pituitary adenomas: a prereceptor determinant of pituitary cell proliferation. *Oncogene 20;22(11):1663-7*

3. **Ayuk J, McGregor EJ, Mitchell RD, Gittoes NJ** 2004 Acute management of pituitary apoplexy - surgery or conservative management? *Clin Endocrinol (Oxf). 61(6):747-52.*

4. **Ayuk J, Clayton RN, Holder G, Sheppard MC, Stewart PM, Bates AS** 2004 Growth hormone and pituitary radiotherapy, but not serum insulin-like growth factor-I concentrations, predict excess mortality in patients with acromegaly. *J Clin Endocrinol Metab 89:1613-1617*

5. **Ayuk J, Stewart SE, Stewart PM, Sheppard MC** 2004 Efficacy of Sandostatin LAR (long-acting somatostatin analogue) is similar in patients with untreated acromegaly and in those previously treated with surgery and/or radiotherapy. *Clin Endocrinol (Oxf) 60:375-381*

Publications linked to this Thesis

1. **Sherlock M, Aragon AA, Reulen RC, Ayuk J, Clayton RN, Holder G, Sheppard MC, Bates A, Stewart PM** 2008 Monitoring disease activity using growth hormone and insulin like growth factor-I in the follow up of 501 patients with acromegaly. *Clin Endocrinol (Oxf) Epub*

2. **Sherlock M, Fernandez-Rodriguez E, Alonso AA, Reulen RC, Ayuk J, Clayton RN, Holder G, Sheppard MC, Bates A, Stewart PM** 2009 Medical therapy in patients with acromegaly: predictors of response and comparison of efficacy of dopamine agonists and somatostatin analogues. *J Clin Endocrinol Metab 94:1255-1263*

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